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THE GROWTH HORMONE GENE CLUSTER: PHYSIOLOGICAL ACTIONS AND REGULATION DURING PREGNANCY

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INTRODUCTION

This manuscript reviews selective aspects of the molecular biology and physiology of the human growth hormone cluster genes during pregnancy. Special emphasis is given to the roles for these hormones in the regulation of fetal growth and metabolism and to the hormonal and other factors that regulate their expression. Particular attention is given to studies from the author's laboratory. Several excellent comprehensive reviews have been published over the past few years that focus on the biology of the individual genes of the cluster.[1,2]

THE HUMAN GROWTH HORMONE CLUSTER

The human growth hormone gene cluster consists of the genes that code for placental lactogen (PL; also known as chorionic

From The Editor's Desk 2009

"You cannot build a house for last year's summer." This Ethiopian proverb may be applicable to the state of *GGH* on its 25th anniversary. Since1984, when the journal was conceived, the editorial board has worked tirelessly to produce a journal of high scientific value, publishing original lead articles and reviews of the most important publications in the field with erudite editorial comments. We have pushed against our deadliness and provided our readers a high quality publication—without commercial bias. It has been gratifying, and *GGH* has become a very well appreciated source of information to pediatric endocrinologists and other specialists interested in the field. We have remained on top of the medical specialty and have been innovative—8 years ago we launched the journal on line. We now reach more than 11,000 subscribers worldwide and almost 500 readers every single day! We have told ourselves, and our readers have acknowledged, it has been *GGH* at its best; and, since it's inception it has been treasured.

However, in the new world of endless headlines and multiple sources of information an educational journal like ours has become difficult to fund. Scientific breakthroughs are published in *The New York Times* and repeated endlessly on cable news. Most scientific journals now contain editorials and review articles and derive strength from the members of the society that funds the journal. They often publish targeted supplements supported by industry. Pharmaceutical companies utilize multiple means to market their products directly to physicians and have turned away from supporting an educational journal like *GGH*, or fallen on hard times themselves. We have made major efforts to continue publishing the journal and have sought support from multiple sources including the pediatric endocrine societies, and industry—to no avail.

We believe that while there is no shortage of information, there is a scarcity of objective, unbiased insight into specific issues in pediatric endocrinology. This has been *GGH's* niche and this is why it should continue informing our very large audience. But our strategy is no longer sustainable as sponsors utilize direct means of reaching and targeting their prospects. Therefore, this *GGH* issue will be the last one of the series that you have enjoyed for 25 years. You will be hearing from us if we are able to obtain the funding necessary to provide you with a valuable unbiased educational resource.

Your thoughts will be welcome at FimaLifshitz@GGHjournal.com.

Respectfully,
Fima Lifshitz, MD
Editor-in-Chief

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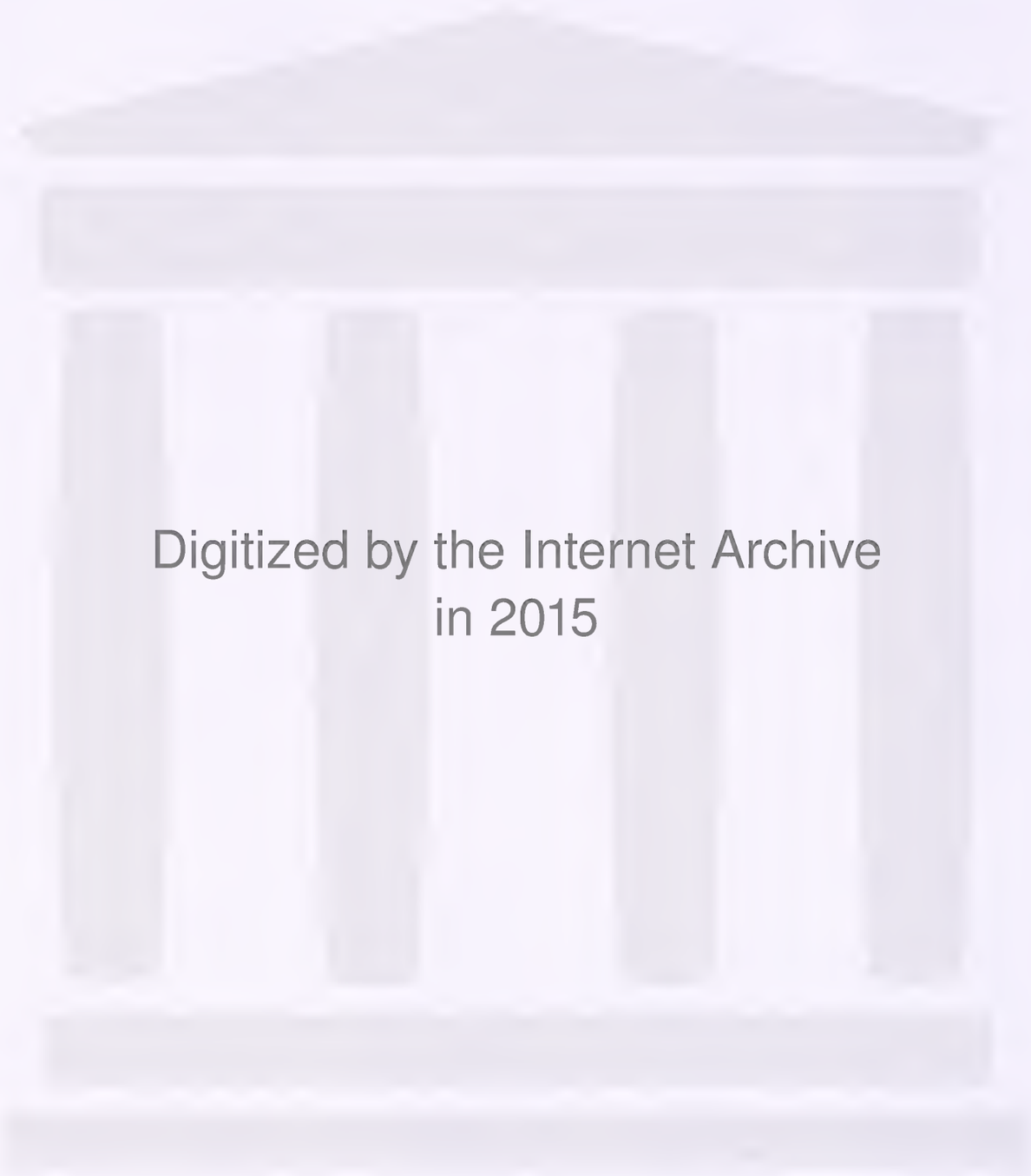

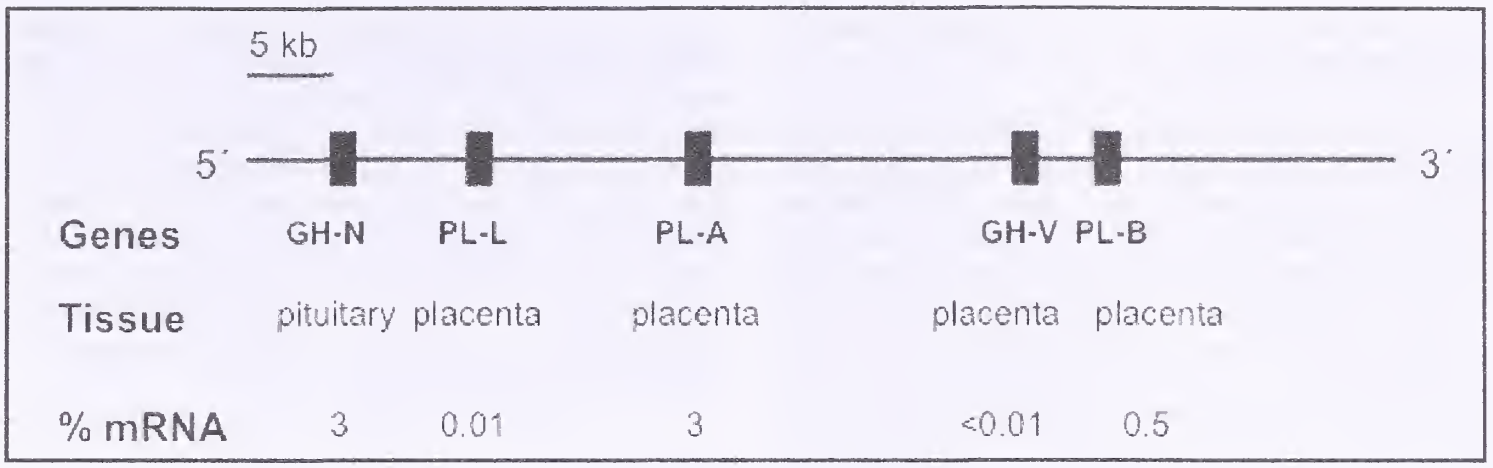

Figure 1. The human growth hormone gene cluster.
The orientation and tissue-specific expression of the five genes comprising the cluster are shown from 5′ to 3′. Also shown is the percentage of total mRNA for each gene in the placenta or pituitary. The abundance of hPL greatly exceeds that of hGH-V.

somatomammotropin, CS), growth hormone variant (GH-V; also known as placental growth hormone) and growth hormone normal (GH-N; also known as pituitary growth hormone). The cluster contains five genes, three PL and two GH genes that evolved from a common ancestral precursor by recombination events involving moderately repeated sequences. The cluster spans 66 kb on chromosome 17 (q22-q24).[3] The individual genes are organized in the same transcriptional orientation and are each composed of five exons and four introns. The order of the genes from 5' to 3' is GH-N, PL-L, PL-A, GH-V, and PL-B (Figure 1). The PL genes and GH-V are expressed exclusively in the placenta, and GH-N is expressed exclusively in the pituitary. As discussed below, the expression of the GH cluster is controlled by a locus control region (LCR) that is located 14.5 to 32 kb upstream of the GH-N gene.[4,5]

PL and GH-V are synthesized and secreted by the trophoblast layer of the placental villus, and the expression of the genes is tightly coupled to placental differentiation. The trophoblast layer is composed of two cell types—mutinucleated syncytiotrophoblast cells and the underlying mononuclear cells that are the precursor cells that proliferate and fuse to form the overlying syncytium.[6] PL and GH-V are not expressed by the cytotrophoblast cells but are expressed as the cells undergo differentiation to a syncytiotrophoblast phenotype. Because of the tight coupling between GH gene cluster expression and villous trophoblast differentiation, the expression of the cluster genes in the placenta is regulated in large part by transcription factors and other signaling molecules that are critical for trophoblast differentiation.

The members of the GH gene cluster share 91% to 99% sequence identities throughout the coding regions and within a 500 bp region immediately upstream of the genes (for review see 7). PL-A and GH-V are alternatively spliced and encode 22 and 26 kD gene products, while PL-B encodes a single 22 kD protein product. The PL-A and PL-B mRNAs are 98% homologous and encode identical mature proteins that are 85% identical to GH-N. The mRNAs for PL-A and PL-B are among the most abundant mRNAs in the placenta, comprising approximately 3.5% of the total mRNA. The PL-A gene is normally expressed at levels three to six times greater than the PL-B gene, probably due to differences in stability of the two mRNAs. The expression of the mRNA encoding PL-L increases towards term;[8] however, the PL-L protein product(s) is not secreted. The amino acid sequence of GH-V and GH-N differ in fifteen positions, thirteen of which are in the mature protein and are distributed throughout the sequence.[9-11] The GH-N gene encodes two alternatively spliced mRNAs that are translated into 22 and 20 kD GH proteins. Although GH-N AND GH-V share striking homologies in structure, immunoassays for GH-N do not detect GH-V and visa versa. Consequently, immunoassays for GH-N cannot be used to measure GH-V.

EXPRESSION OF PL AND GH-V IN NORMAL AND PATHOLOGIC PREGNANCIES

The maternal concentrations of PL and GH-V increase markedly during pregnancy. Human PL is first detected in syncytiotrophoblast cells at 5-10 days after implantation and in maternal plasma at about six weeks of pregnancy.[12] Its concentration then increases linearly until weeks 32-35 of gestation when peak concentrations of 5000 to 7000 ng/mL are attained.[13] The secretion rate near term is about 1.0 gm/day, a rate considerably greater than that of any other polypeptide hormone. Throughout pregnancy, the plasma concentration of PL in the mother correlates with placental mass and is greater in multiple than in singleton gestations. In addition, the pattern of PL secretion during pregnancy roughly parallels the marked increase in maternal plasma insulin-like growth factor (IGF)-I concentrations that normally occurs in pregnancy. Direct measurement of the plasma concentrations of PL in the human fetus in vivo reveals a rise in fetal PL levels from a mean of 5 ng/mL at 20 weeks of gestation to a mean of 20-30 ng/mL at birth.[14] Since radiolabeled PL does not cross the placenta from the maternal

to the fetal circulations, PL appears to be secreted directly into fetal blood.

Aberrations of PL secretion have been detected in many common pathologic conditions of pregnancy, including diabetes mellitus, pre-eclampsia and hypertensive vascular disease.[15-18] In one large series of patients, a single PL concentration below 4 mcg/mL in the last five weeks of pregnancy was associated with 30% risk of fetal distress or neonatal asphyxia. Low PL concentrations on two separate occasions during the last five weeks were associated with a fetal risk of 50% and low concentrations on three occasions with a risk of 71%.[19] In another series of patients, PL concentrations below 4 mcg/mL were detected in 47 of 98 pre-eclamptic patients.[20] Perinatal mortality in the neonates born to the mothers with low PL concentrations was 13% and intrauterine growth retardation was noted in 57% of the neonates.

The lower than normal plasma concentrations of PL and other placental hormones in pregnancies complicated by intrauterine growth retardation (IUGR), pre-eclampsia and other pathologic conditions are probably due in large part to the placental hypoxia and decreased placental mass that is usually found in these conditions. In pre-eclampsia, for example, there is shallow invasion of cytotrophoblast cells into the endometrium, myometrium and spiral arteries of the uterus that results in decreased exchange of substrates, oxygen, hormones and other factors across the placenta. Consequently in pre-eclampsia, IUGR and other pathologic conditions of pregnancy associated with decreased PL, there are multiple factors that contribute to the growth failure of the fetus; and it is not possible to determine the relative contribution of decreased PL concentrations to the growth retardation.

GH-V is first detected in the maternal circulation at about 10 weeks of pregnancy, reaching a maximum in the third trimester of approximately 20-60 ng/mL, becoming the predominant form of GH in maternal serum throughout the latter half of pregnancy.[21,22] GH-V is not detected in fetal serum at any time during pregnancy,[21] indicating that the effects of the hormone on fetal metabolism or growth must be mediated indirectly through actions on maternal and possibly uteroplacental tissues. In contrast, the fetal circulation contains abundant amounts of GH-N, the levels of which rise to a maximum at mid gestation (33.6 ± 2.1 ng/mL by periumbilical blood sampling)[23] with a slow decline to levels approximating 20 ng/mL at term. There is a positive correlation between GH-V concentrations and the birth weight of the fetus; however, GH-V levels in the late second trimester or early third trimester are not predictive of fetal birth weight. Higher GH-V levels have been reported in pregnant women carrying female fetuses, suggesting a gender influence. Both GH-V and PL levels are increased in multiple pregnancies.

Mittal and co-workers have shown that preeclampsia is associated with higher concentrations of placental growth hormone in both the maternal and fetal circulations compared to normal pregnancy.[24] They have also shown that patients with preeclampsia plus small for gestational age (SGA) have lower maternal serum concentrations of GH-V than preeclampsia patients without SGA. Little is known about the regulation of GH-V production in abnormal pregnancies. However, recent studies demonstrate that maternal GH-V levels are reduced in pregnancies associated with IUGR.[22,23,25]

In contrast, GH-N concentrations during gestation remain relatively stable at 4 to 6 ng/mL. Fetal PL concentrations near term are 80 to 125 ng/mL, while GH-V is not detected in fetal plasma. Although GH-N levels remain low in the maternal circulation during pregnancy, GH-N is detected at relatively high concentrations in the fetus. GH-N concentrations in fetal plasma at term are 28 to 38 ng/mL, significantly greater than those detected in maternal plasma.

PHYSIOLOGICAL ACTIONS OF PL AND GH-V

Both PL and GH-V bind to sommatotrophic and lactogenic receptors on a wide variety of tissues and have biological actions in many tissues, including liver, bone, blood cells and placenta. The potency of GH-V in growth-promoting assays is about 7-fold greater than that of PL; and the lactogenic potencies of the two hormones are comparable to that of prolactin. The rise in IGF-I levels in response to the placental hormones likely induces growth of maternal tissues, including the uterus, breast, and thyroid gland. Actions on the heart and kidney may increase cardiac output and maternal blood volume. Recent studies have shown that hGH-V regulates the invasion of extravillous trophoblast cells in the uterus,[26] but it is not known whether PL acts in an autocrine or paracrine manner to regulate placental development and/or function.

Maternal intermediary metabolism undergoes striking changes during pregnancy. In early and mid-gestation, body fat accumulates, while, in mid- to late gestation, the sensitivity to insulin declines and the mother develops postprandial hyperglycemia, hypertriglyceridemia and hyperinsulinemia. Prolonged fasting in late pregnancy leads to exaggerated production of free fatty acids and ketone bodies. These adaptations are thought to insure the continuous supply of glucose and amino acids to the fetus, thereby promoting fetal growth. Several lines of evidence strongly suggest that PL and GH-V play important roles in the metabolic adaptation to pregnancy. PL increases food intake and stimulates glucose uptake, glucose oxidation and the incorporation of glucose into glycogen, glycerol and fatty acids in isolated rat adipocytes, facilitating lipid and glycogen accumulation in the mother in early and mid-pregnancy pregnancy and during the fed state. PL, in concert with prolactin, progesterone, glucocorticoids

and other hormones, reduces insulin sensitivity and induces carbohydrate intolerance in vivo,[27,28] and stimulate ^{3}H-thymidine incorporation, insulin gene transcription, insulin production and glucose-dependent insulin secretion in pancreatic islet cells.[29,30] These actions of PL therefore contribute to postprandial hyperglycemia and hyperinsulinemia in the pregnant mother in mid to late pregnancy. PL also increases the basal rates of lipolysis in adipocytes and the plasma concentrations of nonesterified fatty acids, ketones and glycerol. The mobilization and utilization of maternal free fatty acids for energy spares maternal glucose for the fetus.

Several lines of evidence suggest that PL also has direct anabolic effects on fetal metabolism that promote fetal growth. PL is present in the fetal circulation in relatively high concentrations, binds to fetal tissues that are critical for fetal growth, and has direct growth-promoting actions on fetal tissues. The administration of PL to hypophysectomized rats increases tibia epiphyseal growth and plasma IGF-I concentrations with a potency approximately 5% to 10% that of GH. Furthermore, the placental hormone stimulates amino acid uptake, DNA synthesis and IGF-I production in cultured human fetal myoblasts, fibroblasts and hepatocytes.[31-33] The effects of PL on ^{3}H-thymidine incorporation and amino acid transport are blunted, though not abolished, by an antiserum to IGF-I, suggesting that the action of PL is mediated in part through the paracrine release of IGF-I. PL and prolactin also stimulate DNA synthesis and insulin production in fetal and neonatal pancreatic explants and promote the formation of islet-like cell clusters in cultured pancreas cells.[30,34,35] These findings strongly suggest roles for PL in the induction of islet cell growth and insulin production in the late-gestational fetus. Other possible roles for PL in the fetus include the production of fetal adrenocortical steroid hormones and development of the fetal lung. PL also stimulates DNA synthesis in human mammary epithelial cells and growth of ductal epithelium, suggesting that the hormone may facilitate mammary development prior to delivery. A summary of the biological actions of PL in the mother and fetus is shown in Figure 2.

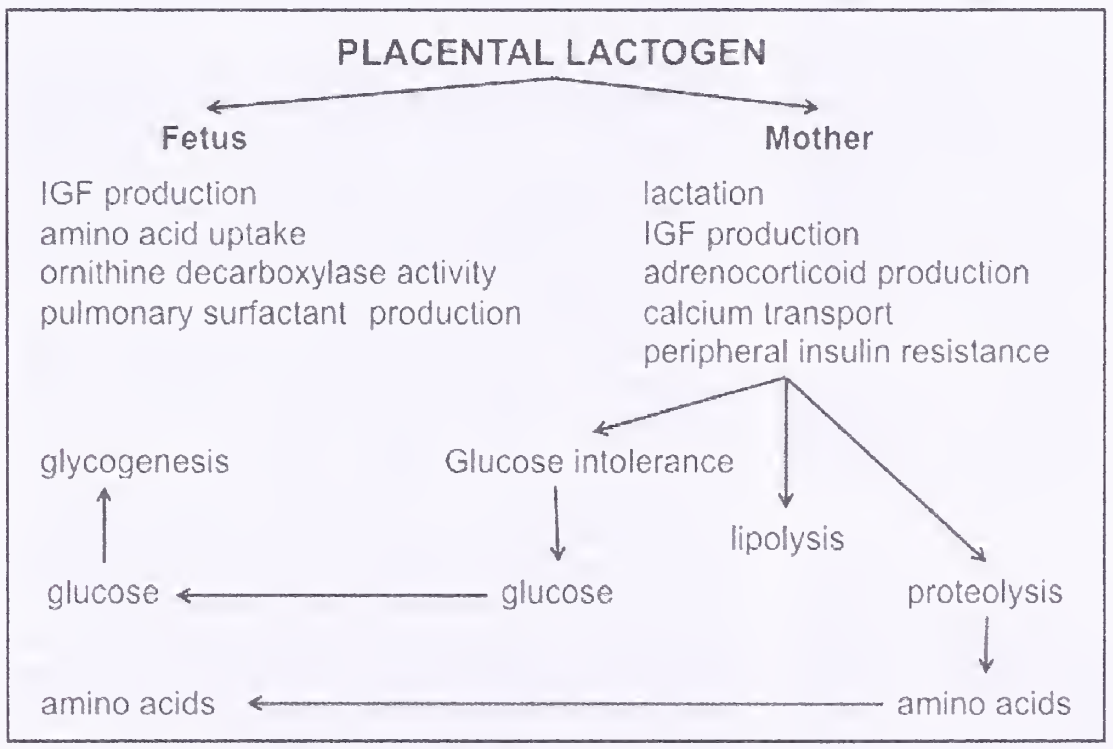

Figure 2. The biologic actions of PL in the mother and fetus.
PL has direct affects on fetal and maternal tissues that modulate fetal growth and metabolism. The induction of peripheral insulin resistance in the mother leads to glucose intolerance with resulting hyperglycemia as well as to an increase in lipolysis and proteolysis. The net effect of these changes is the transport of glucose and amino acids to the fetus and the stimulation of glycogenesis and protein synthesis in the fetus. PL also induces IGF production in the mother and fetus and stimulates lactation in the mother and pulmonary surfactant production in the fetus. The enzyme ornithine decarboxylase is important for the synthesis of DNA, RNA and protein.

On the other hand, several lines of evidence indicate that GH-N plays only a limited role in fetal linear growth. Patients with isolated GH deficiency, pituitary aplasia or anencephaly have only minimal or modest (and inconsistent) reductions in birth length.[36] Furthermore, a deficiency of GH in experimental animals has little or no effect on fetal growth. For example, dwarf mice deficient in pituitary GH have normal tail lengths at birth and modest (14%) reductions in birth weight, though serum IGF-I and IGF-II concentrations are reduced significantly.[37] Decapitation, encephalectomy or hypophysectomy of fetal rabbits, rhesus monkeys, rats, mice, or pigs is not accompanied by fetal growth failure or reductions in serum IGF-I concentrations, and electrolytic destruction of the ovine fetal medial-basal hypothalamus with concomitant GH deficiency has no effect on fetal plasma IGF-I or IGF-II concentrations (reviewed in 38). Conversely, an excess of fetal GH is not accompanied by fetal overgrowth.

Although GH-N may have only a limited effect on the longitudinal growth of the fetus, the hormone appears to have important effects on fetal metabolism and development. For example, clinical experience substantiates a role for GH-N in perinatal carbohydrate metabolism. The neonatal hypoglycemia may result in part from heightened sensitivity to insulin; however, deficient storage of glycogen in fetal liver may also play a role because GH stimulates glycogen synthesis and inhibits glycogenolysis[39] in

isolated hepatocytes from fetal sheep and fetal rats. GH also stimulates DNA synthesis and IGF-I production in isolated human fetal hepatocytes as well as DNA synthesis, insulin production and glucose-dependent insulin secretion in isolated pancreatic islets from human adults and fetal and neonatal rats and mice. These latter observations implicate a role for GH in perinatal islet development and function. The high prevalence of micropenis in newborn males with GH deficiency or GH resistance[40,41] implicates a role for pituitary GH in the regulation of human phallic growth in utero.

REGULATION OF THE HUMAN GROWTH HORMONE GENE CLUSTER

As discussed below, the GH cluster is controlled by a remote LCR that is located 14.5 to 32 kb upstream of the GH-N gene.[4,5] The LCR contains five hypersensitive sites (HSI-HSV), which are short regions of chromatin detected by supersensitivity to cleavage by DNase 1. These sites, which are only found in active genes, appear before the initiation of transcription and are generated as a result of the binding of transcription factors that displace histones after binding to DNA within the hypersensitive site. Closely linked HSI and HSII are pituitary specific, HSIV is placental specific, and HSIII and HSV are present in both tissues. HSV and HSIII, at -32 kb and -28 kb, are detected in pituitary somatotrope and placental syncytiotrophoblast cell chromatin; HSIV, at -30 kb, is specific to syncytiotrophoblast cell chromatin; and HSI and HSII, at -14.5 kb to -15.5 kb, are specific to somatotrope chromatin. The GH LCR and the GH-N promoter are encompassed by a continuous 32 kb pituitary-specific domain of acetylated histones H3 and H4 with a central peak located at HSI. Histone acetylation is linked to transcriptional activation; and histone acetyltransferases (HATs) and histone deacetylases (HDACs) are recruited to promoters through physical interaction with sequence-specific transcription factors. Site-specific inactivation of HSI results in loss of acetylation throughout this domain, loss of critical transfactor occupancy at the GH-N promoter, and a 20-fold reduction in GH-N expression. Thus, HSI plays an essential role in the establishment of the acetylated domain and in activation of GH-N transcription in the pituitary.

Histone acetyltransferase (HAT) activity recruited to HSI establishes a continuous 32 kb domain of histone acetylation connecting the LCR and the GH-N promoter. This acetylated domain facilitates transfactor binding at the GH-N promoter and transcriptional activation of GH-N. Activation of the placental genes in the term placental syncytiotrophoblast cells is marked by activating histone modifications that are restricted to HSV-HSIII and to the placental genes; the regions between, which include HSI,II and the GH-N gene, remain unmodified. Based on the present knowledge of cellular differentiation and epigenetic alterations, it seems reasonable to propose that chromatin structures in the placenta are altered during the terminal transition from cytotrophoblast cells to syncytiotrophoblast cells to result in robust induction of gene expression from the GH cluster.

Gene activation during cytotrophoblast cells differentiation to a syncytiotrophoblast cell phenotype is initiated by H3K4 methylation of HSIII-HSV of each individual placental gene repeat (PGR) unit.[42] Subsequent transcriptional activation is accompanied by acetylation of histones H3 and H4 encompassing the entire placenta-expressed region of the cluster. The distribution and progression of chromatin modifications suggests that each PGR independently initiates transcription. Initial activating chromatin modifications are nucleated within the individual PGR units; and subsequent transcriptional induction relies on additional determinants and more extended chromatin modifications.

REGULATION OF PL AND GH-V EXPRESSION

Although in vivo studies have provided information about the regulation of PL and GH-V secretion, most information about the expression of these hormones has been obtained using primary cultures of human cytotrophoblast cells or explant cultures. The primary cytotrophoblast cells, which are prepared by enzymatic dispersion of term or pre-term placental tissue, undergo spontaneous aggregation, syncytialization and terminal differentiation and express genes normally expressed by syncytiotrophoblast cells, including PL and GH-V.

Using these in vitro model systems, many factors have been shown to induce trophoblast differentiation and the expression of PL and GH-V. These factors include epidermal growth factor,[43] chorionic gonadotropin,[44] leukemia inhibitory factor,[45] colony stimulating factor-1,[46] IGF-I,[47] cyclic AMP,[48] members of the transforming growth factor β superfamily,[49] the Wnt/β-catenin pathway,[50-52] the transcription factors PPARγ,[53] Ikaros,[54] GATA-2/3,[55] and several other factors in the differentiation process. Oxygen has also been shown to be a critical factor in the differentiation process and the induction of the GH cluster genes (for summary see 56). Low oxygen tension directs placental differentiation along the extravillous trophoblast cell pathway in which cytotrophoblast cells invade the uterus. Greater oxygen tension directs differentiation along the villous trophoblast cell pathway and the formation of the trophoblast layer that lines the placental villus. Recent studies from the author's laboratory have also demonstrated a critical role for the transcription factor TFAP2A (also known as AP2, activator protein 2) in syncytiotrophoblast formation and the induction of PL and GH-V. [57,58]

Knockout experiments in the mouse have identified many transcription factors that are important in the differentiation of the various cell types constituting the murine placenta,[59-61] including HOXB6, HOXC5, HOXC6,

HOX3E, HB24, GCM1, GAX, MSX2, DLX4, Pit-1, HAND1, TF-1, TEF5, c-Ets1 and several other transcription factors, many of which are helix-loop-helix (bHLH) proteins. ID-2, a member of a family of inhibitors of bHLH binding, acts in trophoblast cells as a dominant/negative bHLH transcription factor;[62] and constitutive overexpression prevents differentiation of the cells. However, the roles for homologs of these transcription factors in human placental development are not known.[63,64]

PL expression

At present, the specific hormonal and metabolic factors that regulate the secretion of PL are incompletely PL understood. Although PL has striking homologies in structure and function to GH-N, the factors that regulate the expression of the two hormones are different. For example, changes in circulating levels of free fatty acid concentrations, amino acids such as arginine, estrogens, oxytocin, prostaglandins, epinephrine, TRH, GnRH, dopamine and glucocorticoids do not effect modulate PL secretion. While changes in blood glucose concentrations modulate GH-N secretion, glucose does not appear to have consistent effects on PL secretion in the mother. Most investigators have failed to demonstrate significant changes in PL concentrations following glucose administration or insulin-induced hypoglycemia. However, a significant decrease in plasma PL concentrations was noted in one study following two intravenous infusions of glucose one hour apart or the continuous infusion of glucose over several hours. Several studies have reported a 30%-40% increase in plasma PL concentrations in women fasted 84-90 hours during weeks 16-22 of gestation (prior to therapeutic abortion[65]). Interestingly, angiotensin II has also been shown to stimulate PL secretion in vitro.[66]

Studies of the regulation of PL gene expression suggest a role for autocrine/paracrine factors in the regulation of PL gene expression. 1,25-dihydroxyvitamin D_3, interleukin (IL)-6 and IL-1, all of which are synthesized and secreted by syncytiotrophoblast cells, stimulate the synthesis and release of PL by trophoblast cells.[67,68] 1,25-dihydroxyvitamin D_3 stimulates PL gene expression via the vitamin D receptor that binds to a composite nuclear hormone receptor site on the PL promoter.[69] Retinoic acid and thyroid hormone also stimulate PL gene expression via the binding of RARA and TRB receptors to the same composite site.[70] The action of IL-6 is mediated, at least in part, by the transcription factor NF-IL6 that binds to three consensus NF-IL6 elements on the distal PL promoter.[71] It is likely that other cytokines and nuclear hormone receptors are also involved in the regulation of PL expression.

Recent studies strongly suggest a novel physiologic role for high density lipoproteins (HDL) in the regulation of PL gene expression during pregnancy.[72] The stimulation appears to be due primarily to pre-β HDL, a minor component of the total HDL in the circulation that is much smaller in size than the major circulating form (α-HDL) but which contains a much higher apolipoprotein (apo) A-I/lipid ratio. During pregnancy, pre-β concentrations in maternal plasma increase markedly with a pattern that parallels that of PL.[54] Pre-β HDL concentrations increase from 3% to 4% of the total HDL in the early first trimester to about 20% at term.

The stimulation by HDL is mediated by apoA-I and, to a much lesser extent, apoA-II and apoC. Amphipathic peptides that mimic the tree dimensional structure of apoA-1 also stimulate PL promoter activity and the expression of PL from cultured trophoblast cells. The action of apoA-1 is due, at least in part, by activation of adenylate cyclace and phospholipase C. ApoA-1 stimulates a time- and dose-dependent increase in MAP kinase activity. ApoA-1 has also been shown to have other non-lipid-dependent effects, including the stimulation of endothelial cell proliferation, endothelin-1 production by renal cells, and the inhibition of degranulation and superoxide dismutase activity in neutrophils. Plasma apoA-I concentrations have been reported to be significantly lower than normal in several pathologic conditions of pregnancy associated with decreased plasma PL concentrations and IUGR, including pre-eclampsia[73] pregnancy-induced hypertension[74] and insulin-dependent diabetes mellitus.[75] Whether the low apoA-I concentrations contribute to the decrease in PL secretion in these patients is unknown.

GH-V expression

The secretion of GH-V, like of GH-N, is induced by hypoglycemia and suppressed by glucose and is regulated by cAMP. However, unlike GH-N, GH-V is released tonically and is not regulated by growth hormone releasing hormone, ghrelin and somatostatin. In addition, the GH-V promoter is not regulated by the transcription factor Pit-1. Studies by Lominick and Handwerger[76] have shown that the GH-V promoter is transactivated by the transcription factors MEF2 and FOXF1 but not FOXF2. Since FOXF1 and FOXF2 bind to the same DNA binding site, the difference in the ability of the two FOX proteins to transactivate the GH-V promoter is likely due to differential binding of the proteins to one or more co-activators.

SUMMARY

The human GH cluster consists of five closely related genes. GH-V, PL-A, PL-V and PL-L are expressed exclusively in the placenta, while GH-N is expressed exclusively in the pituitary. Both PL and GH-V have growth-promoting and lactogenic activities during pregnancy. PL is detected in both the maternal and fetal circulations and has direct growth-promoting actions in both compartments. GH-V, on the other hand, is only detected in the mother. Although PL and GH-V have striking structural and biological homologies to GH-N, the

factors that regulate the expression of placental genes are different from those that regulate pituitary growth hormone. Aberrations in PL and GH-V have been noted in several pathologic conditions of pregnancy, including preeclampsia and IUGR.

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Letter to the Editor

Disorders of Sex Development: Nomenclature

The European Society for Paediatric Endocrinology (ESPE) published a classification of pediatric endocrine diagnoses in 2007. Diagnoses made by pediatric endocrinologists were divided into 14 groups, including *Disorders of Sex Development* (DSD). DSD were subdivided into the categories of Sex Chromosome DSD; 46,XY DSD; 46,XX DSD; and Unclassified Forms of Abnormal Sexual Development/Anatomical Disruptions. The impetus for this letter is the exclusion by ESPE of "disorders of gonadal differentiation that do not result in sex reversal/virilised female infant/undervirilised male" from the category of Sex Chromosome DSD. Specific examples of conditions excluded are Klinefelter syndrome and Turner syndrome, both of which are instead classified under the general category of Syndromes with Endocrine Features (subcategory of Chromosomal Abnormalities).

Although a comment on a nomenclature first published in November 2007 may seem overdue, the dilemma that the ESPE DSD classification system has created remains unresolved. To the best of our knowledge, the points we raise have not previously been enunciated, and the issue remains every bit as problematic as when the new nomenclature was first published.

In 2005, working groups, comprised of 50 international experts (including Sandberg and Vilain), members of the Lawson Wilkins Pediatric Endocrine Society (LWPES) and ESPE, assembled in Chicago to formulate a consensus document on the clinical management of individuals born with intersex conditions.[1] One of the working groups focused on nomenclature and several significant changes were adopted by the whole consensus group. The most visible modification to the previous nomenclature recommended was the removal of terms perceived as offensive such as "hermaphrodite" and "pseudohermaphrodite," and the change of "intersex" –a politically charged and somewhat vague term–to DSD. Yet two additional profound changes were implemented. One was to incorporate all aspects of sexual variations under one umbrella term (DSD) defined as "congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical." This allowed doing away with the simplifying notion that gonads are the only parameter defining sex. The other major modification was to remove references to gender in the diagnostic nomenclature in order to avoid gender labeling–often psychologically disturbing to the patient.

ESPE's revised classification of Sex Chromosome DSD contradicts the nosology endorsed just two years earlier at a meeting co-sponsored by ESPE itself. A group of distinguished international clinical and scientific experts from a large variety of fields (genetics, endocrinology, psychology, psychiatry, surgery) and representatives of patient support groups participated in a long and complex process involving preparation of draft documents prior to the consensus meeting, working group, and general discussions during the meeting, group writing of the consensus statement, and multiple post-meeting edits. It is unclear why one party to a consensus agreement, representing only one subspecialty (pediatric endocrinology), from one region of the world (Europe), would unilaterally modify the product of an International Consensus Group which had painstakingly considered the complex issues of nosology for DSD.

The principle guiding exclusion from Sex Chromosome DSD (ie, "disorders of gonadal differentiation that do not result in sex reversal/virilised female infant/undervirilised male") implies that atypical genital appearance is the sine qua non of DSD. If we follow the argument that Turner and Klinefelter syndromes should not be classified as DSD because the external genitalia are normal, we should also exclude from DSD women with XY pure gonadal dysgenesis–who have normal external genitalia, males who are XX caused by a translocation of SRY, who often have normal male genitals, and even Complete Androgen Insensitivity Syndrome (CAIS), who appear at birth with normal female genitalia. One of the reasons why the term "intersex" was set aside was its vague meaning. Intersex implied sexual ambiguity, yet every physician agreed that CAIS was encompassed by the term intersex.

In addition, the nomenclature adopted at the International Consensus Conference was designed to overturn the practice of classifying DSD exclusively based on the characteristics of the gonads, which did not reflect the various parameters influencing sexual development. The definition of DSD now includes not only the gonads and the genitals, but also the sex chromosomes as a parameter.

Furthermore, excluding Klinefelter syndrome from the subcategory of sex chromosome DSD because it does not result in "undervirilised males" is questionable and depends on one's definition of "undervirilised." Suggesting that small, dysgenetic testes, which do not support spermatogenesis—a major male function—are not undervirilised seems to be a subjective interpretation.

Finally, the ESPE document uses the word "sex reversal" that was clearly abandoned in the consensus statement because of its uncertain meaning, but reemerges in the ESPE document.

By using an argument based exclusively on the appearance of the external genitalia to eliminate Klinefelter and Turner from sex chromosome DSD, the ESPE classification implicitly undermines the value of the DSD nomenclature introduced in the consensus statement by weakening the inherent logic behind the classification system, which is about multiple aspects of sexual development, and not exclusively focused on the appearance of the genitals and the issue of gender assignment.

An argument favoring the removal of Klinefelter and Turner syndromes from the category of DSD is articulated by the editors in the foreword of the ESPE classification, where they note that "we have tried to follow the logic of the paediatric endocrine clinician as much as possible, so that it would be as easy as possible to find the diagnosis in the structure of each chapter." However, they also state that the coding system should "follow one general principle (e.g. nosology, aetiology, pathogenesis or symptomatology)." The editors have followed both standards: the latter, principle-driven, by embracing the term DSD and its definition, and the former, practitioner-friendly, by inserting Klinefelter and Turner syndrome in a different section where it has traditionally been found. The classification of DSD could indeed be entirely based on clinical phenotype and clinician observations. Turner syndrome could then be classified with XY gonadal dysgenesis and CAIS, based on the appearance of the external genitalia. This would discount recent advances in the understanding of DSD, which are crucial in outcome and prognosis studies. Classifications and nomenclatures evolve with science, and the comfort of practicing endocrinologists should be balanced with the realities of biology and the specific needs of our patients. This is why Turner and Klinefelter syndromes, which are clear disorders of sexual development, undoubtedly belong within the DSD classification.

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REVIEWS & COMMENTS FROM THE LITERATURE

Another Cause of Primary IGF Deficiency

Primary insulin-like growth-factor deficiency (PIGFD), abnormally low levels of IGF-I despite normal or elevated levels of growth hormone (GH), has been attributed to mutations in 4 genes to date: *GHR*, *IGF1*, *STAT5b*, and *IGFALS*. *IGFALS* encodes the acid-labile subunit (ALS) of the ternary complex, also under GH control. Fofanova-Gambetti et al reported 2 patients with 3 novel mutations in *IGFALS*, plus another 2 patients in the amendment while the paper was in press, to add to the currently published tally of 5 patients from 3 families harboring 4 different mutations. Of note, in contrast to patients with mutations of the other PIGFD genes, all patients with *IGFALS* mutations presented with modest short stature (height z-scores above –3 SD).

Previously published patients:

Case 1: A boy aged 14.6 years from Argentina with a height z-score of –2.05 SD and homozygous *IGFALS* mutation1338delG (E35fsX120), in the amino terminal flanking region.[1]

Case 2: A Turkish boy aged 12.1 years with a height z-score of –2.9 SD and homozygous *IGFALS* D440N missense mutation in the 17th leucine-rich repeat (LRR) domain.[2]

Cases 3-5: Three Norwegian/German siblings (2 male, 1 female) aged 15.3 to 19.6 years, with height z-scores of –0.5 to –2.0 SD and compound heterozygous C540R/583_591dup9 *IGFALS* mutations in the cystein-rich region of the carboxy terminus and the 7th LRR domain, respectively.[3]

Currently reported patients:

Case 1: A boy of 6.7 years of Mayan origin with a height z-score of –2.91 SD, delayed bone age (5.5 years) and homozygous *IGFALS* 1308_1316 dup9 mutation in the 17th LRR domain. GH treatment began at the age of 8.5 years and was discontinued 1 year later due to development of nonalcoholic steatotic hepatitis. The patient's transaminase levels continued to climb when he was off treatment, however they subsequently returned to normal. GH therapy was tried again from age 10 years for another 2 years. Despite increasing doses of GH, he failed to improve his growth velocity or normalize his IGF-I and IGF binding protein (IGFBP)-3 levels. During this time, at the chronological age of 10.5 years, he initiated spontaneous puberty and was started on LH-RH analogue therapy to preserve growth potential while on GH. At age 12 years, he was switched from GH to IGF-I therapy.

Case 2: A girl aged 4.1 years of Eastern European Jewish/Icelandic-Western European ethnic origin with a height z-score of –2.14 SD, bone age consistent to her chronologic age, and compound heterozygous *IGFALS* C60S/L244F missense mutations in the 1st and 9th LRR domains, respectively. She started GH treatment at age 4.4 years, increasing her height z-score in 13 months to –1.67 SD; IGF-I and IGFBP-3 levels nonetheless remained abnormally low, and ALS was undetectable.

Patients reported in the amendment:

Case 1: An Indian/Pakistani boy aged 15.2 years with a height z-score of –3.17 SD, delayed bone age (11 years), sexual infantilism and homozygous *IGFALS* L134Q missense mutations in the 4th LRR domain. His parents, both heterozygous carriers, had normal heights (–0.09 and –1.35 SDS).

Case 2: An Ashkenazi Jewish boy aged 12.7 years with a height z-score of –2.87 SD, bone age of 11.5 years, sexual infantilism and compound heterozygous *IGFALS* P73L/L241P missense mutations in the 1st and 8th–9th LRR domains, respectively. His parents, both heterozygous carriers of one of the mutations, had normal heights (–1.68 and +0.85 SDS).

ALS protein, a member of the LRR superfamily of proteins involved in protein-protein interactions, contains 20 LRR domains that form a donut shape with a closed structure. The LRRs contain β-strands that form sheets inside the donut, and α-helices that flank the structure's outer circumference. This paper highlights the ethnic and genetic heterogeneity of *IGFALS* mutations that are pathogenic in causing PIGFD and modest short stature that responds poorly to GH therapy. Although GH can induce IGF-I and IGFBP-3 production, without ALS, circulating levels of the growth factor are not sustained. This is a nice in vivo illustration of the importance of the ternary complex in prolonging the circulating half-life, and hence activity, of IGF-I.

Fofanova-Gambetti OV, Hwa V, Kirsch S, et al. Three novel IGFALS gene mutations resulting in total ALS and severe circulating IGF-I/IGFBP-3 deficiency in children of different ethnic origins. Horm Res. 2009;71:100-110.

Editor's Comment: *Genotyping of the parents of Girl #2 in this paper was not available. The authors hypothesized that her mutations must be in the compound heterozygous state because her ALS protein was undetectable; had her mutations occurred in cis, then her wild-type allele would be expected to produce wild-type ALS that should have been detected, as was the case for the carrier parent of the Turkish boy with a homozygous missense mutation.[2] Another possibility is that the double mutations in cis so altered the ALS protein product that it functioned as a dominant negative, tying up the wild-type ALS in the ER or Golgi and preventing its secretion. This second hypothesis would require that one of the parents similarly carry the dominant negative in cis mutations, have undetectable ALS, and be affected. The father's height z-score was +0.30 SD while the mother's was –2.13 SD. Since one of the main teaching points of this paper is that ALS mutations cause PIGFD with only modest short stature, perhaps the mother is affected like her daughter?*

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Acute Vascular Effects of GH Appear to be Independent of Both Local and Systemic IGF-I Production

Growth hormone (GH) has been shown to regulate vascular tone and reactivity in humans, but it is unclear whether this action is a result of a direct stimulatory effect of GH or if it is dependent on systemic and local insulin-like growth factor (IGF)-I production. In this study, Li et al evaluated the mechanisms underlying the acute vascular effects of GH. Ten healthy lean young volunteers (20 to 27 years of age; 7 male and 3 females) were studied after an overnight fast. GH was infused for 6 hours at 0.06 mcg/kg/minute and a biopsy of the vastus lateralis muscle was

obtained in 7 of these subjects before and after infusion for analysis of IGF-I mRNA and Akt phosphorilation. Blood was obtained serially every 10 minutes during the infusion for GH, IGF-I, insulin and glucose assessments. GH infusion increased plasma GH and forearm blood flow by 66% ($p<0.001$), but did not change plasma IGF-I concentrations, muscle IGF-I mRNA expression, or muscle Akt phosphorylation–therefore suggesting a lack of IGF-I action in muscle. Additionally, human aortic endothelial cells (HAECs) were incubated with GH (30 ng/mL) in vitro for 3 or 6 hours. GH did not alter endothelial nitric oxide synthase (eNOS) protein content, but induced a time-dependent increase of the phosphorylation of eNOS. This study demonstrated that GH exerts an acute vascular effect, independent of both systemic and local IGF-I production and that this effect probably occurs via direct action on GH receptors and eNOS in the vascular endothelium.

Li G, del Rincon P, Jahn LA, et al. Growth hormone exerts acute vascular effects independently of systemic or muscle insulin-like growth factor I. J Clin Endocrinol Metab. 2008;93:1379-1385.

Editor's Comment: *Endothelial dysfunction appears to explain much of the increased cardiovascular risk of GH deficiency. GH seems to play an important role in the regulation of peripheral vascular resistance and vascular reactivity; these effects appear to be mediated by the activation of the NO pathway. GH deficiency is associated with decreased systemic NO formation and decreased forearm release of nitrite and cyclic GMP during acetylcholine stimulation, as well as a decreased peak hyperemic response to ischemia, which reverts to normal during GH replacement. Significant endothelial dysfunction–as determined by an impaired endothelium-dependent brachial artery dilatory response to occlusion ischemia and by abnormalities of several biochemical markers of endothelial cell activation–has been reported in adolescents and adults with GH deficiency.[1,2] It is not clear whether these effects are a result of a direct effect of GH on the vascular endothelium or whether they are dependent on systemic and local IGF-I production. This study seems to indicate that the acute vasodilatory effect of GH is exerted independent of IGF-I, very possibly through GH receptor mediated eNOS activation.*

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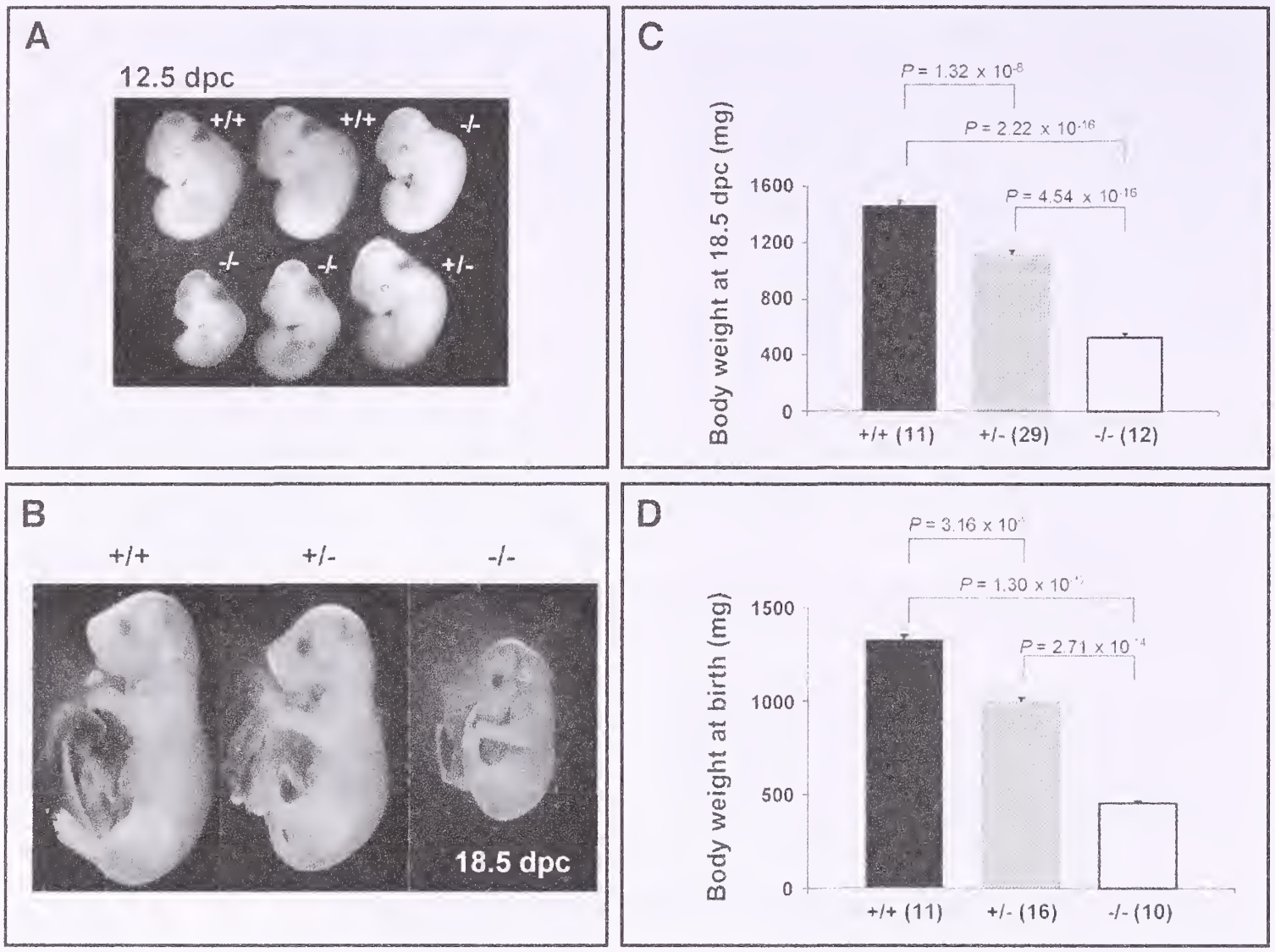
Nedd4 Controls Animal Growth by Regulating IGF-I Signaling

Nedd4 (Neural precursor cell expressed developmentally down regulated 4 - OMIM 602278, chromosome 15q) is a cytoplasmic ubiquitin ligase that regulates protein movement and structure thereby its function or directs a protein into ubiquitin-proteasomal degradative pathway. Cao et al demonstrated that Nedd4 is essential for transduction of intracellular signals initiated by insulin and insulin-like growth factor (IGF)-I and the localization of the insulin receptor (*IR*, OMIM 147670, chromosome 19p13.2) and the IGF-I receptor (*IGF1R*, OMIM 147370, chromosome 15q25-q26) to the cell plasma membrane. Nedd4 does not bind to IR or IGF1R directly, but links to an adaptor protein, Grb10 (Growth factor receptor-bound protein10, OMIM 601523, chromosome 7p12-p11.2), which in turn is bound by IR and IGF1R. Grb10 inhibits movement of these receptors to their localization sites in the plasma membrane and thereby impairs function of IR and IGF1R. This effect is opposed by the binding of Nedd4 to Grb10. Cao and colleagues generated Nedd4 knockout (KO) mice. $Nedd4^{-/-}$ mice died during gestation or shortly after birth due to immature lung development and aeration (Figure); their linear growth and weight were severely impaired by embryonic day 12.5. Heterozygous $Nedd4^{-/+}$ mice were also small at birth and through post-natal age 3 months (the end of the study period). In vitro, the proliferation of $Nedd4^{-/-}$ fibroblasts was impaired relative to that of wild-type fibroblasts due to decreased progression through the cell cycle at phases G_0 and G_1. IGF-I and insulin mediated intracellular signaling was substantially reduced in *Nedd4*$^{-/-}$ and *Nedd4*$^{+/-}$ fibroblasts and could be restored by expression of Nedd4 in these cells. However, in *Nedd4*$^{-/-}$ fibroblasts, the expression and translation of IR and IGF1R were normal, but the receptors did not reach the cell surface, an abnormality that could also be reversed by expression of *Nedd4* in these cells. Further studies demonstrated that the amount of Grb10 was increased in *Nedd4*$^{-/-}$ fibroblasts and that "knockdown" of Grb10 by small interfering RNA (siRNA) restored insulin and IGF-I signaling in *Nedd4*$^{-/-}$ fibroblasts. The investigators concluded that Nedd4 positively regulates IGF-I and insulin signaling by enhancing the movement of their receptors to the cell surface. Nedd4 does so by dis-inhibiting the inhibitory effect of Grb10 on this process—perhaps by controlling the rate of degradation of Grb10 itself through the ubiquitin-proteasomal system.

Cao XR, Lill NL, Boase N, et al. Nedd4 controls animal growth by regulating IGF-1 signaling. Sci Signal. 2008;1:ra5. [DOI:10.1126/scisignal.1160940]

Editor's Comment: *This study has identified another intracellular signal transduction site (Nedd4-Grb10) to examine when a patient with severe growth retardation due*



Nedd4$^{-/-}$ mice die immediately after birth, and *Nedd4*$^{+/-}$ and *Nedd4*$^{-/-}$ mice exhibit intrauterine growth retardation. No mice homozygous for disruption of the *Nedd4* gene were found 2 or 3 weeks after birth. Ratios of heterozygotes and homozygous mutants were thus assessed at earlier time points: (A) 12.5 dpc, (B and C) 18.5 dpc, and (D) immediately after birth. Both heterozygotes and homozygous mutants showed signs of intrauterine growth retardation as early as 12.5 dpc (A) and at late gestation [18.5 dpc (B) and (C)]. At the time of birth [postnatal day 1 (D)], the body weights among three genotypes differed significantly: *Nedd4*$^{-/-}$ body weight averaged 64 to 68% lower relative to that of wild-type littermates; heterozygote body weight averaged about 15 to 20% reduction in body weight relative to that of wild-type littermates. In (C) and (D), the numbers of animals used for the analyses are shown in parentheses; the body weight was significantly different between groups of mice, with P values indicated.

to insensitivity to IGF-I and an intact IGF1R is encountered. A polymorphic variant or mutation in either one of these proteins might also account for impaired intrauterine growth in some small-for-gestational age neonates.

Allen W. Root, MD

Growth Hormone Deficiency: Transient or Permanent?

In this multicenter study, Berberoglu and colleagues tried to assess the need for continuation of growth hormone (GH) treatment in adulthood after growth is completed and also to evaluate factors that would predict persistent GH deficiency (GHD). A total of 70 (31 female, 39 male) GHD patients were included in the study; 52 patients (74%) had isolated GHD and 18 patients (26%) had multiple pituitary hormone deficiency (MPHD). The initial diagnosis was based on a peak GH level <10 ng/mL in 2 pharmacological tests. GH treatment was discontinued in these patients when growth velocity during the previous year decreased to less than 2 cm and the bone age had reached greater than 14 years in girls, and greater than 16 years in boys, and after completion of puberty. All patients were re-tested by insulin tolerance test (ITT) at least 6 weeks after discontinuation of the replacement treatment. Serum insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3 concentrations were determined at the same time. If GH peak during ITT was <3 ng/mL, the patient was diagnosed to have severe permanent GHD.

Among the patients with isolated GHD, 9 patients

(17.3%) were found to have persistent GHD and 43 (82.7%) to be transiently GH deficient. On the other hand, among patients with MPHD only 2 patients (11.1%) were transiently GH deficient.

None of the parameters differed significantly with respect to gender. There were significant positive correlations between peak GH and IGF-I, and IGFBP-3 levels in all patients (IGF-1 r=0.297, p=0.036; IGFBP-3 r=0.45, p=0.03). The IGF-I and IGFBP-3 SDS values were lower in the group that had peak GH values <3 ng/mL. When the cut-off was taken as –2 SD, specificity and sensitivity of IGF-I in confirming persistency of GHD were 65.7% and 73.3%, respectively. Its positive predictive value and negative predictive value were 33.3% and 85.2%, respectively. For IGFBP-3, specificity and sensitivity were 84%, and 60%, respectively. The positive and negative predictive values were 60%, and 84%, in the same order. Finally, while the negative predictive values were high for both of these parameters, an IGFBP-3 value below –2 SD was found to be more specific than an IGF-I value below –2 SD.

The data in this study confirmed that there were no auxological and clinical signs to predict the transiency or the persistence of GHD except for a history of organic disease and presence of MPHD. The authors concluded that most patients with childhood onset GHD were idiopathic and GHD was frequently transient in this group of patients. In contrast, GHD was persistent in patients with MPHD. They emphasized the high negative predictive values for IGF-I and IGFBP-3 (85.1% and 84%, respectively) suggesting that normal IGF-I and IGFBP-3 levels highly exclude the diagnosis of GHD.

Berberoglu M, Siklar Z, Darendeliler F, et al. Evaluation of permanent growth hormone deficiency (GHD) in young adults with childhood onset GHD: a multicenter study. J Clin Res Ped Endo. 2008;1:30–37.

Editor's Comment: *The question of how to confirm the diagnosis of adult GHD in an adolescent patient who has completed linear growth is still being debated. The Growth Hormone Research Society guidelines suggest a peak GH response on ITT of <3 ng/mL as being diagnostic of GHD in adulthood.[1] Although patients with MPHD have peak GH levels <3 ng/mL, it is not clear whether this value can confirm adult GHD exactly. In addition, despite the high negative predictive values of IGF-I and IGFBP-3, the use of serum IGF-I and IGFBP-3 alone to predict GHD cannot be recommended. The majority of children with GHD, when retested as adults, do not have the classical severe GHD.[2] This high incidence (70%) of normal GH responses on retesting has been shown in patients with idiopathic and isolated GHD.[3] This finding indicates that the organic etiologies are often severe and can be assumed to be permanent at the beginning of the therapy. Therefore, those patients with organic MPHD could be excluded from retesting.*

The patients who have peak GH cut-off values between 3-5 ng/mL might be GH deficient as well. In fact, in the transition period in late adolescence a cut-off value of 5 ng/mL is advocated for the diagnosis of persistent GHD and continuation of GH therapy because adolescents have higher GH levels than adults. In this study, there were 3 additional patients with peak GH level between 3-5 ng/mL in the isolated GHD group and none in the MPHD group. Therefore, no suggestion is available for patients in this gray zone. Furthermore, the prognosis of patients with a GH response of 5-10 ng/mL is not known. Therefore, it is important to keep in mind that clinical signs of GHD may occur later in life and the clinician must look for these manifestations in patients with a history of childhood GHD.[3]

Ömer Tarim, MD

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IGFBP-3 Promoter Polymorphism Affects Response to GH Treatment for GH Deficiency

Growth responses to growth hormone (GH) therapy vary considerably among children with GH deficiency despite receiving standardized per kg body weight doses. Several clinical factors have been identified in influencing responsiveness to treatment,[1] but about half of the variation remains unexplained. These clinical factors only indirectly consider genetic traits, by including parental target heights.

Thus, Costalonga et al sought to examine the effects of an *insulin-like binding protein* (*IGFBP*)*-3* promoter polymorphism on growth velocity during the first year of GH treatment in prepubertal children with severe GH deficiency. In twin studies, about 60% of the interindividual variability in circulating IGFBP-3 levels was found to be genetically determined.[2] A single nucleotide change 202 bp upstream of the transcription start site was found to affect IGFBP-3 promoter activity in vitro and in vivo; mean circulating IGFBP-3 levels in healthy adults were highest in those with AA genotype at the –202 position, less in AC and lowest in those with CC.

Costalonga et al studied 48 boys and 23 girls with severe GH deficiency (mean height z-score of –4.3 ± 1.4 SD,

mean bone age delay of 4.3 ± 2.7 years, and peak GH response in 2 stimulation tests ranging from <0.1 to 3.3 mcg/L). All children were prepubertal with a mean age of 8.6 ± 4.1 years, and treated exclusively with GH at a mean dose of 32 mcg/kg/day adjusted to weight every 3 to 4 months. Seventeen percent of subjects had a defined genetic etiology for GH deficiency, 63% had ectopic posterior pituitary and 25% had interrupted stalk on MRI imaging; only 8% had idiopathic GH deficiency, and patients with central nervous system tumors, meningoencephalocele or previous radiation therapy were excluded from the study.

Among the 71 subjects, 21% had –202 *IGFBP3* genotype of AA, 54% had AC, and 25% had CC. The genotype subgroups did not differ clinically at the start of treatment, nor in mean GH treatment doses. Mean circulating IGFBP-3 levels also were not significantly different at baseline, they gained significance with GH treatment; AA subjects had higher IGFBP-3 levels than C allele carriers in codominant (P<0.005) and recessive models (P<0.001), and developed greater increases in IGFBP-3 z-scores with treatment. The *IGFBP3* polymorphism accounted for 19% of variability in circulating IGFBP-3 levels (P<0.001) and 54% of variability when combined with age and gender.

The *IGFBP3* polymorphism did not associate with IGF-I levels either at baseline or during GH treatment, but it did affect growth response to treatment. Mean first year growth velocity was 13.0 ± 2.1 cm/year in AA subjects, 11.4 ± 2.5 cm/year in AC subjects, and 10.8 ± 1.9 cm/year in CC subjects (P<0.05). Single and multiple linear regression analyses found the effect of *IGFBP3* polymorphism independent of other variables in associating with growth velocity. It accounted for 10% of variability in growth velocity (P<0.005) and 29% of variability when combined with height z-score and age at start of treatment.

This is the first study of the –202 A/C *IGFBP3* polymorphism in children. Because the genotype was significantly associated with circulating IGFBP-3 levels in healthy adults and in children with severe GH deficiency only after GH treatment but not at baseline, the authors concluded the effect is at least in part dependent on GH action.

Costalonga EF, Antonini SR, Guerra-Junior G, Mendonca BB, Arnhold IJ, Jorge AA. The -202 A allele of insulin-like growth factor binding protein-3 (IGFBP3) promoter polymorphism is associated with higher IGFBP-3 serum levels and better growth response to growth hormone treatment in patients with severe growth hormone deficiency. J Clin Endocrinol Metab. 2009;94:588-595.

Editor's Comment: *This study conveys 2 important lessons. First, the results may seem counter-intuitive: the genotype associated with the highest IGFBP-3 levels had the greatest growth response to GH treatment. The IGFBPs were defined by their high-affinity IGF binding that renders them competitive inhibitors for IGF binding to the type 1 IGF receptor (IGF1R), and hence inhibitors of IGF action.[3] This is an isolated effect. The situation in vivo and some in vitro cell models is more complex, because the balance of ligand binding protein receptor and post-receptor signaling pathways is modulated by multiple factors. Such factors include, but are not limited to, changes in ligand half-life, local IGFBP proteases that convert the high-affinity IGF binders to lower affinity IGFBP fragments, IGF1R trafficking and down-regulation, and interactions with other cell signaling systems. Plus, we now appreciate that the IGFBPs exert IGF-independent actions of their own.*

Secondly, this study highlights yet another factor that influences patient responsiveness to GH treatment. I applaud the authors' focus on clearly defined subjects with severe GH deficiency, rather than opening up their sample size to less severe and thus, heterogeneous, patients who may harbor other alterations in their GH/IGF axis function. The authors concluded their paper with the suggestion that future pharmacogenetic studies may support adjusting GH treatment to genotype in order to individualize and thereby optimize therapy. Before moving to genotyping–which is expensive and not readily available–clinicians already have tools to individualize therapy. For example, titrating GH dose to achieve desired IGF-I z-scores, as the principle mediator and biomarker of GH effects, is akin to titrating l-thyroxine dose to thyroid function tests when treating patients with hypothyroidism.[4] This paper provides additional data supporting the notion that the traditional, cookie cutter, one-size-fits-all, weight-based dosing of GH therapy can be improved by individualized approaches to optimize treatment efficacy and safety.

Adda Grimberg, MD

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GH Treatment for Growth Failure in Pediatric Patients with Crohn's Disease

Heyman and colleagues studied the effects of growth hormone (GH) treatment (0.043 mg/kg/day; 0.3 mg/kg/week) on height velocity, body composition, and disease activity in a group of children and adolescents (mean age 12.6 ± 4.5 years; 6 males) with Crohn's Disease (CD) and growth failure. All

subjects had a confirmed endoscopic, histological, and/or radiographic diagnosis of CD and height below the 5th percentile for age with no evidence of catch-up growth (increase in height z-score of 0.5) for the year prior to GH therapy. Exclusion criteria included hepatic abnormalities, renal disease, history of non-compliance, and pre-existing scoliosis. Subjects were seen at baseline and every 3 months for 12 months for a history, physical assessment of anthropometric measurements, calculations of BMI and body fat mass, as well as laboratory studies to evaluate disease activity. Nutritional state, serum vitamin B12, iron levels, red blood cell folate, plasma insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3 were measured at each visit and the CD activity was characterized using the Pediatric CD Activity Index (PCDAI). Bone age was determined by wrist radiography. Bone density and body composition were assessed using DEXA at the lumbar spine (L1 to L4) and hips. Age adjusted values were used for comparison and variation of z-scores. The comparison control group was gathered from the PEDI IBD Consortium Registry which included consecutively enrolled patients from 6 sites with inflammatory bowel disease; 989 children were identified as having CD. For each subject receiving GH, 3 comparison subjects with CD were retrospectively matched by age, sex, race, and height (at baseline). The patients in the control group were receiving standard treatment and nutritional supplementation for CD.

The study group had a mean bone age of 10.7 years with an average diagnosis of CD for 2.7 years, PCDAI of 21.9, a height z-score of –2.48, and a weight z-score of –1.88 with a previous year's growth velocity of 2.8 cm/year. The control group had a similar age, and a mean height z-score of –1.8 with a mean weight z-score of –1.19. Each patient remained on his or her clinically indicated therapy for CD which included temporary total parenteral nutrition (TPN), elemental formula diet, or regular diet. All subjects consumed more than 85% of the RDA of calories for age. BMI did not increase significantly from baseline at 12 months, however DEXA scans at 1 year of GH treatment demonstrated an increase in mean lumbar z-scores and a decrease in mean percent body fat; the bone age increased by 0.97. IGF-I level increased from 249.4 ± 146.8 to 447.1 ± 242.6 at the end of treatment. Mean IGF-BP3 was within the range adjusted normal range. No significant changes in thyroid functions, or electrolytes were observed. Mean height velocity increased from 3 ± 1.39 cm/year at baseline to 8.32 ± 3.2 cm after 1 year of GH. Within the control group the mean height velocity was 3.98 ± 2.32 cm/year at baseline and 4.84 ± 2.85 cm/year after 1 year; this difference was significant. The height z-score increased by 0.76 and the weight z-score increased by 0.81 as compared with increases of 0.16 and 0 in the control group. The mean PCDAI was 21.9 at baseline and 13.1 after 1 year of treatment. No subject experienced any adverse reaction to GH. Two patients were excluded from the comparison, one of whom had a disease exacerbation requiring 2 hospitalizations during the 12 month study period and the other due to a lack of a matched comparison.

The authors stated that their data suggest that children with CD treated with GH experience increased height velocity and improved bone mineral density. There have been 10 other pediatric inflammatory bowel disease uncontrolled GH trials. Results from these studies have varied, but they have included small numbers of subjects and no disease controls. The authors noted that despite the increase in growth, there was no consistent clinical improvement in CD activity. Thus, it would appear that GH is not a primary treatment strategy for CD. They also noted the limitations of having used a retrospective comparison group and the small size of their study, which prohibited controlling for concomitant medications, including corticosteroids and other supplements. They concluded that a larger randomized trial of GH therapy in CD is needed.

Heyman MB, Garnett EA, Wojcicki J, et al. Growth hormone treatment for growth failure in pediatric patients with Crohn's Disease. J Pediatr. 2008;151:651-658.

Editor's Comment: *The authors reported that growth impairment is seen in about 40% of pediatric patients with CD and that this often leads to short stature in adulthood. The possible etiology of this growth failure may include anorexia, inflammation, direct effects of cytokines on bone, GI nutrient losses, GH resistance with low IGF-I and other medications including corticosteroids. Of note, growth impairment may precede the onset of intestinal symptoms in CD.*

Pediatric endocrinologists recognize the importance of looking for inflammatory bowel disease when evaluating children with short stature. Indeed CD is occasionally diagnosed during the evaluation for short stature prior to any GI symptoms. The authors clearly pointed out that other studies have shown variable results when GH is used to treat short stature and growth failure in CD and the limitations of those studies.

Studies of growth impairment in complex disease states such as CD may provide information on the importance of a variety of different disease processes associated with growth failure. In other words, are inflammatory processes critical or is the effect of cytokines on bones critical? Thus a study of a large number of individuals for whom assessments of these factors have been well characterized may lead to an important understanding of growth, not just in CD, but in other chronic disease processes.

William L. Clarke, MD

Pathogenesis of Hypothalamic Obesity in Children

The pathogenesis of hypothalamic obesity is not clear. In this multicenter study, the investigators studied the role of leptin, soluble leptin receptor (sOb-R), resistin, and insulin secretory dynamics in the development of hypothalamic obesity. Children who had hypothalamopituitary tumors were divided into 2 groups. The first group included obese-overweight (hypothalamic obese [HOB] group, n=23) and second group included non-obese children (hypothalamic non-obese [HNOB] group, n=16). Exogenously obese-overweight children (OB group, n=22) were included as controls. Oral glucose tolerance test (OGTT), basal serum leptin, sOb-R, resistin levels, and homeostasis model assessment (HOMA) indexes were compared between the groups. Age, sex, and pubertal status were similar in study groups. Median and interquartile ranges of BMI z-scores were similar in HOB and OB groups.

The ratio of the patients who received chemotherapy and radiotherapy were similar in the 2 groups. Tumor size, relapse rates, and number of operations were not different between the groups. The number of patients with multiple pituitary hormone deficiency as well as ACTH, TSH, GH, ADH, and gonadotropic hormone deficiencies were also similar in HOB and HNOB groups. Growth hormone (GH) replacement dose was 0.025–0.035 mg/kg/day for the patients with GH deficiency, and hydrocortisone replacement dose was ≤10 mg/m^2/day in all patients with central adrenal insufficiency. All patients with central hypothyroidism were receiving adequate replacement dose of L-thyroxine to maintain free T_4 levels in the normal range.

	HOB Group	HNOB Group	OB Group
Leptin/BMI	4.0 (1.6–5.2)	1.5 (0.8–3.1)	2.5 (1.8–3.5)
Leptin/sOb-R (FLI)	2.0 (0.8–3.5)	0.6 (0.3–1.2)	1.5 (1.0–2.3)

Serum leptin levels corrected for BMI were highest and total leptin/sOb-R ratios (free leptin index [FLI]) tended to be higher in HOB than HNOB and OB groups, indicating leptin resistance (Table). Serum resistin levels were similar in all groups. Basal serum glucose, basal and second-hour insulin levels in OGTT, and HOMA index were higher in OB group than the HOB and HNOB groups, indicating insulin resistance in simple obesity; however, the increment of insulin to the same glycemic load in OGTT was highest in the HOB group indicating insulin dysregulation ($p<0.05$). It was concluded that hypothalamic obesity seemed to be related to both dysregulated afferent (leptin) and efferent (insulin) neural outputs through the autonomic nervous system resulting in energy storage as fat.

Guran T, Turan S, Bereket A, et al. The role of leptin, soluble leptin receptor, resistin, and insulin secretory dynamics in the pathogenesis of hypothalamic obesity in children. Eur J Pediatr. 2008; Nov 29 [Epub ahead of print]

Editor's Comment: *Hypothalamic obesity is a frustrating syndrome which develops following an insult to the hypothalamic area.[1,2] The pathophysiology of this condition is not clear and therefore therapeutic attempts usually fail. There may be many confounding factors which may affect body weight and energy homeostasis all of which have been more or less controlled in this study.*

In both HOB and HNOB groups, only the leptin levels were remarkably higher in the tumors with hypothalamic/thalamic involvement (p values 0.023 and 0.01, respectively). The findings of higher leptin/BMI and higher FLI in hypothalamic patients suggest the contribution of leptin resistance in the pathogenesis of hypothalamic obesity. A recent study by Shaikh et al also confirmed that hyperleptinemia is associated with obesity following hypothalamic damage in children.[3]

The primary defect in patients with hypothalamic obesity is believed to be altered neural regulation of the beta-cell secretion resulting in insulin hypersecretion, in contrast with simple obesity, where peripheral insulin resistance is assumed to be the primary defect driving a compensator beta-cell response. In agreement with this hypothesis, this study shows that HOMA index representing insulin resistance is higher in the common obese groups compared to the patients with brain tumors in the hypothalamo-pituitary region. This finding implies the importance of dysregulated insulin secretion to a glycemic load rather than insulin resistance in the development of hypothalamic obesity differently from exogenous obesity.

In conclusion, compared to simple obese children, HOB patients have lower HOMA and lower basal insulin, but a higher insulin response to a glycemic load and higher leptin/BMI. These findings support that in hypothalamic obesity there are both dysregulated afferent (leptin) and efferent (insulin) neural outputs through the autonomic nervous system resulting in energy storage as fat. Dysregulated insulin secretion, rather than insulin resistance, is characteristic of hypothalamic obesity. Obviously, more studies are needed to further elucidate the mechanisms of hypothalamic obesity in order to offer more rewarding therapies for the patients.

Ömer Tarim, MD

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Hypovitaminosis D In Obese Children

Recent studies have reported a relation between obesity and vitamin D hypovitaminosis.[1] In this cross-sectional study, Çizmecioğlu et al aimed to determine the prevalence of vitamin D hypovitaminosis in a highly industrialized city in the Marmara region of Turkey where obesity is on the rise. At the first stage of the study, anthropometric measurements of 2491 subjects participating in the research were performed in the schools. At the second stage, participants whose BMI was over the 85th centile were invited to the hospital for further investigation. A total of 301 students (177 girls, 124 boys) aged 11 to 19 years were selected by multistage stratified sampling design. Children with any systemic disease or using any medications or supplements known to affect skeletal metabolism were excluded from the study.

Of the 301 children and adolescents who were included in the study, 102 were obese (34%) and 145 were overweight (48%). BMI values were within normal percentile ranges in 54 (18%) who had lost weight and returned to normal BMI when the blood samples were collected for further investigation. Serum 25-hydroxyvitamin D (25-OHD), intact parathyroid hormone (iPTH), and alkaline phosphatase (ALP) were measured in late winter months. Vitamin D deficiency was defined as a 25-OHD <10 ng/mL, insufficiency as 25-OHD 10 to 20 ng/mL, and normal vitamin D level as >20 ng/mL.

The prevalence of hypovitaminosis D was 65% in all students (12% deficiency and 53% insufficiency). Vitamin D deficiency in female students was about 2 times more common than in males. None of the girls were veiled in this study. Although the girls appeared to have higher BMI values than the boys, there was no statistically significant difference between their BMI SDS values. There was also no relation between obesity status and vitamin D categories. However, there was a negative correlation between serum vitamin D level and BMI in obese and overweight subjects whose vitamin D level <20 ng/mL (r: – 0.186 $p<0.01$). There were no correlations between serum 25-OHD and ALP and iPTH levels.

The authors concluded that vitamin D deficiency and insufficiency were common in obese and overweight schoolchildren, especially in girls, and obesity could be a risk factor in adolescents.

Çizmecioğlu FM, Etiler N, Görmüş U, Hamzaoğlu O, Hatun Ş. Hypovitaminosis D in obese and overweight schoolchildren. J Clin Res Ped Endo. 2008;1:89–96.

Editor's Comment: *The same authors previously reported high rates of subclinical vitamin D deficiency (65%) in adolescent girls (from the same region) who wear concealing clothing.[2] This study shows that a veil is not the only factor responsible for hypovitaminosis D. Air pollution that may block ultraviolet light may also contribute to the lack of sun exposure in this highly industrialized city. Indeed, the rate of vitamin D deficiency was higher in industrialized towns compared to the rural area in this study. However, it was shown that among students who live in the same area, serum 25-OHD levels decreased as BMI increased suggesting a causative role of obesity as well. The authors argued that this inverse relationship was consistent with the hypothesis suggesting that the increased adipose tissue decreases vitamin D bioavailability by sequestration in body fat.[3] Unfortunately, there is no information about the dietary intake of these patients and the effect of reduced ingestion of micronutrients such as iron and vitamin D in obese people is not taken into account. The duration of sun exposure could not be assessed either, but it was assumed that industrialized areas would be exposed to less sunlight because of blockage by air pollution. In this study, the cut-off for vitamin sufficiency was taken as 20 ng/mL, in contrast to many other studies where the cut-off is more appropriately suggested at 30 ng/mL or even 40 ng/mL. Nevertheless, vitamin D levels were studied as a continuous variable and regression analysis revealed the inverse relationship between BMI and serum vitamin D level.*

Other studies have also reported that inadequate vitamin D intake was associated with obesity in young adults.[4] Infants with rickets are also known to be chubby. Reverse causality may also be suggested, and whether or not vitamin D deficiency per se causes obesity remains to be investigated. However, the possible role of isolated hypovitaminosis D in causing obesity is a difficult issue to be studied in humans since vitamin D deficiency may be associated with other nutritional deficiencies which may lead to malnutrition rather than obesity.

Whether obese children and adolescents require a higher dose of vitamin D supplementation is controversial. As the authors stated, cross-sectional design and the limited number of subjects were limitations of this study. Further longitudinal studies are necessary to define the role, if any, of hypovitaminosis D in the etiology of obesity and the dose and duration of vitamin D supplementation in childhood, particularly in the obese and overweight population.

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Pediatric Obesity: Meta-Analysis of Non-Surgical Interventions

In order to inform practice guidelines, the Endocrine Society's Task Force on Pediatric Obesity engaged the Mayo Knowledge and Encounter Research Unit to conduct a meta-analysis of published, randomized trials for pediatric obesity lifestyle and pharmacological interventions.[1] Overweight or obese children aged 2- through 18-years served as participants in the individual studies forming the meta-analysis. Pharmacological interventions included medications aimed at reducing measures of obesity in children (ie, BMI, percent overweight, percent fat-free mass and visceral adiposity). Lifestyle interventions included treatment strategies targeting physical activity and/or dietary changes. Eligible treatments targeted the child, parent, family, school, or community. Interventionists included community agents, school personnel, family members, or healthcare personnel.

Fully published randomized trials were identified through a systematic search of the following databases: MEDLINE, EMBASE, ERIC, CINAHL, Cochrane Central Register of Controlled Trials, PSYCInfo, Dissertation Abstracts International, Science Citation Index, and Social Science Citation Index. Publications through February 2006 were included. Reference sections of reviews and published guidelines were reviewed, and suggestions from experts on The Endocrine Society Pediatric Obesity Task Force were included. From these, 76 articles were considered eligible for the meta-analysis; in all, 61 trials had complete data to include in meta-analyses. Working in pairs, trained reviewers extracted study details and mean or variance data were calculated.

Effect size and 95% confidence interval (CI) for the difference between the intervention and control groups were calculated, as well as standardized mean differences. Subgroup analyses were conducted for degree of parental participation, child age, percent body fat versus BMI, and the combination of reduced sedentary behavior and increased physical activity. Standardized mean differences of about 0.2 or less were considered small, about 0.5 as moderate, and about 0.8 or greater as large effect sizes. The likelihood of between-study variability being attributable to true between-study differences (versus chance) was quantified using the I^2 statistic (inconsistency is considered small when I^2 <25%, moderate 25%-50%, and large >50%).

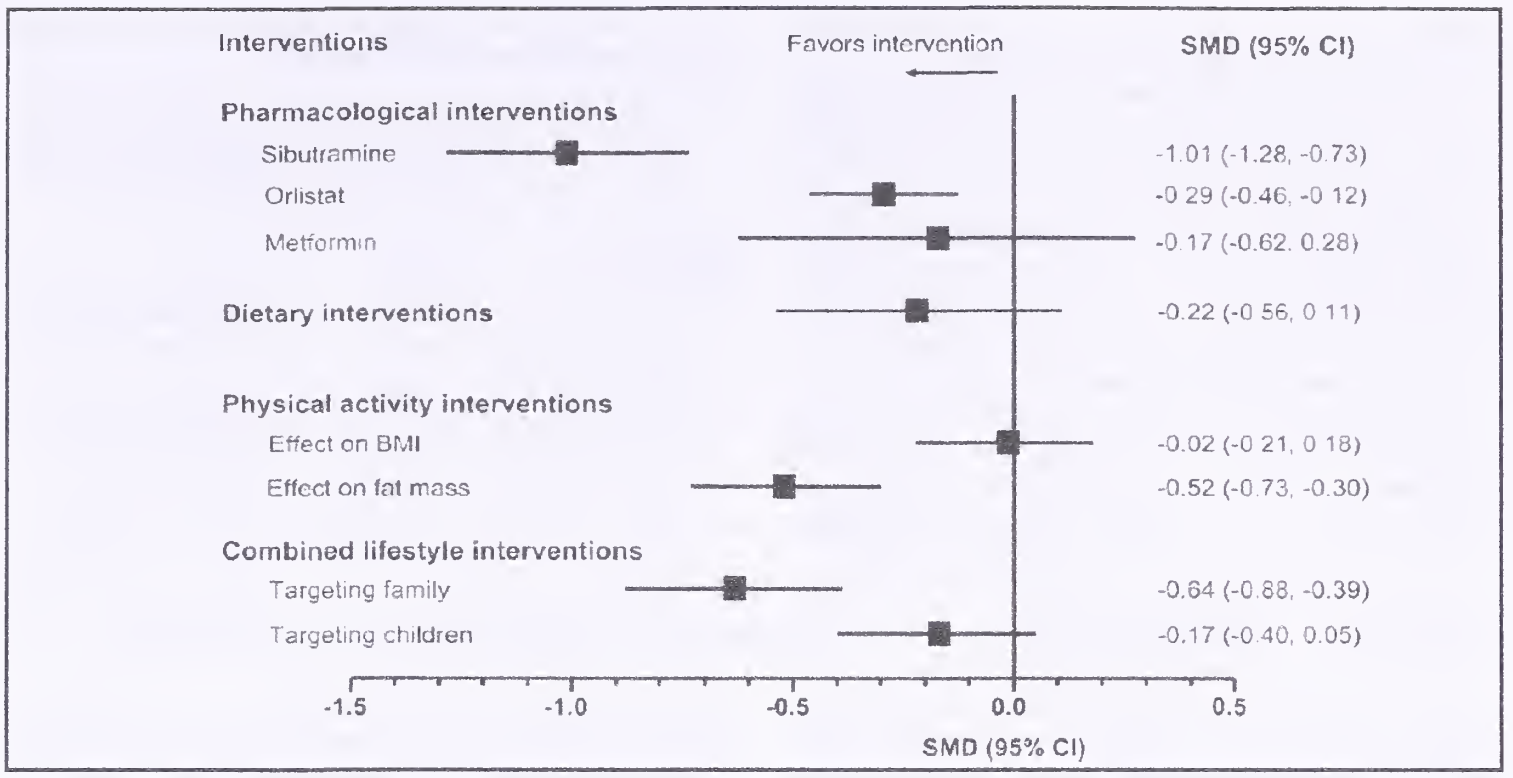
A total of 17 trials of pharmacological interventions formed this portion of the meta-analysis: sibutramine (3 trials)— the pooled effect size, favoring treatment, was large (–1.01; CI = –1.8 to –0.73; I^2 = 30%) and consistent with a loss in BMI of 2.4 kg/m^2 (CI = 1.8 to 3.1 kg/m^2) after 6 months of use (patients taking sibutramine had higher rates of elevated blood pressure and pulse rate than patients taking placebo); orlistat (3 trials) – the pooled effect size was small to moderate (–0.29; CI = –0.46 to –0.12; I^2 = 0%) and consistent with a loss in BMI of 0.7 kg/m^2 (CI = 0.3 to 1.2 kg/m^2). More patients taking orlistat reported GI side effects than patients on placebo; metformin monotherapy for hyperinsulinemic nondiabetic obese adolescents lead to a small nonsignificant change in obesity outcome at 6 months (–0.17; CI = 0.62 to 0.28). The remaining trials measured the effect of sympathomimetics (ephedrine and caffeine, dexfenfluoramine), dehydroepiandrosterone, and fiber supplements (results reported in figure). Trials of rimonabant in children or adolescents were not identified in the literature.

Lifestyle interventions were divided into "dietary interventions only" (ie, reduced-glycemic-load diet, protein-sparing modified diet, low-carbohydrate diet, high-protein diet, and hypocaloric diet versus control; n=6), "physical activity interventions only" (n=17), and "combined lifestyle interventions" (n=23). The pooled effect sizes and between-study inconsistency for dietary interventions were both small (–0.22; CI = –0.056 to 0.11; I^2 = 22.5%). Physical activity interventions yielded inconsistent results: the investigators examined whether the choice of obesity outcome measure accounted for this. Trials that measured effects on adiposity found a moderate treatment effect (–0.52, CI = –0.73 to –0.30; I^2 = 0%), whereas trials measuring the effect of physical activity on BMI found no significant effect (–0.02, CI = –0.21 to 0.18; I^2 = 0%).

The pooled estimate across combination lifestyle interventions (physical activity and dietary modification) yielded small to moderate treatment effects. The largest effects were associated with greater parental involvement. There was a nonsignificant interaction between child age and parental involvement, with a trend toward a larger treatment effect for children 8 years or younger (–0.70; CI = –1.00 to –0.40).

The authors concluded: (1) short-term efficacy of sibutramine and orlistat on BMI; (2) moderate effect of physical activity on adiposity, but not BMI; and (3) small to moderate effect of combined lifestyle interventions on BMI with a nonsignificant trend favoring those interventions with parental involvement, in particular trials involving younger children. The authors discussed research implications related to drawbacks associated with using BMI as an outcome measure (eg, less responsive to change, requires accuracy and reproducibility in measurement, and misinterprets risk in muscular and short children), and suggested using more responsive outcome measures such as fat-free mass or percent body fat in future studies. The authors also suggested that the Endocrine Society's recommendation for a multidisciplinary and multimodal approach to the treatment of pediatric obesity be studied with long-term randomized trials.

McGovern L, Johnson JN, Paulo R, et al. Treatment of pediatric obesity: A systematic review and meta-analysis of randomized trials. J Clin Endocr Metab. 2008;93:4600-4605.



Overall summary of meta-analyses results of randomized trials of treatments for pediatric obesity.
Plot shows metanalytic estimates (■) and 95% CI (*horizontal lines*). SMD, standardized mean differences.

Editors' Comment: *The generally dim view of the effectiveness of non-surgical approaches to the management of pediatric obesity may be an important contributing factor to the increasing visibility of bariatric surgery programs. The current meta-analysis provides valuable information for the clinician, clinical and public health researcher with an interest in ensuring that management moves forward in an evidence-based manner. Meta-analysis provides the benefit of increasing the statistical power of small or inconclusive studies and can demonstrate how interventions deliver heterogeneous effectiveness in different settings and in different patients. The benefits do not come without drawbacks, however. Meta-analysis cannot improve the quality of the original studies. Further, aggregation of studies without sufficient attention to the heterogeneity of procedures may result in misleading conclusions.*

In the case of the current meta-analysis, there was substantial variability in the procedures adopted across lifestyle intervention trials. These studies varied greatly with respect to duration of the intervention, frequency of sessions, content of dietary, behavioral, and exercise components, training of the interventionists, and measurement. For example, in trials categorized as "combined lifestyle intervention category," some used the Traffic Light Diet, whereas others did not state specifically which nutrition recommendations were offered. Duration of exercise also varied; some interventions held exercise sessions 3-times per week, whereas others only provided exercise education. Furthermore, specification of the specific behavioral strategies employed during treatment to promote behavior change is not consistently described. Given the high degree of unspecified details across studies, it would be premature to adopt generalizations regarding the value of lifestyle interventions, per se.

Effect sizes of the interventions included in this meta-analysis were estimated in reference to control groups. Not only was there great variability across procedures adopted as interventions, there was also great variability with respect to how control groups were defined. Some researchers chose a no-treatment control group, whereas others altered aspects of the intervention delivered to the treatment group and used this modified intervention as the control group. The strength of findings for any single study may be diminished when the intervention group is compared to a control group that is also offering some form of the intervention, albeit modified. The high degree of variability across interventions, together with highly variable control groups, leads us to question whether a meta-analysis is premature.

Another factor to be considered when interpreting the findings of this meta-analysis includes categorization of the lifestyle interventions. Six studies were designated "dietary interventions only." Closer examination of these reveals that 5 of the 6 studies included behavioral and/or physical activity components in addition to the dietary intervention. Only one study was exclusively dietary.[2] Similarly, for the 17 studies designated as "physical activity interventions only," 8 also included a dietary and/or behavioral component. Although the focus of the studies in these categories may be primarily dietary or physical activity, the addition of other components may weaken the conclusions that can be drawn from a meta-

analysis that is specifically analyzing "dietary interventions only" or "physical activity interventions only."

An additional detail regarding studies classified as "combined lifestyle interventions" is noteworthy. The authors suggested a statistically non-significant trend for a larger treatment effect in interventions involving parental involvement in children 8 years of age or younger. This conclusion was based on only 2 studies in which the majority of participants were under age 8 years. Moreover, one of these studies examines a parent-only approach to weight management.[3]

Given some of our observations, it might be premature to draw firm conclusions about the magnitude of effect sizes of dietary or behavioral interventions and their variability across populations. The obvious risk would be to make pronouncements that bias future research agenda.

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Prevention of Pediatric Obesity: Meta-Analysis of Behavioral Interventions

The prevalence of overweight (ie, BMI >95th percentile for age) is currently 16% in children of all ages living in the US. The highest rate occurs among African-American youth. The Endocrine Society's Task Force on Pediatric Obesity commissioned a meta-analysis of published, randomized trials for interventions aimed at preventing pediatric obesity.[1] In contrast to previous summaries of the literature that focused on the endpoint of body weight, this study sought to summarize the efficacy of interventions aimed at changing lifestyle behaviors, including increased physical activity (PA), decreased sedentary activity (SA), increased healthy dietary habits (HD), and decreased unhealthy dietary habits (UD) to prevent pediatric obesity. In addition, the investigators sought to assess the effect of these interventions on BMI.

Studies eligible for inclusion in the meta-analysis were randomized controlled trials (RCTs) assessing these lifestyle behavior interventions in children or adolescents 2 to 18 years of age. Participants received the interventions at home, school, clinic, or a community setting and healthcare professionals, community members, or health authorities delivered the interventions. Trials with participants who were all overweight or obese were excluded.

Fully published randomized trials were identified through a systematic search of the following databases: MEDLINE, ERIC, EMBASE, CINHAL, PSYCInfo, DISSERTATION abstracts, Science Citation Index, Social Science Citation Index, and the Cochrane CENTRAL database of Controlled Clinical Trials. Publications through February 2006 were included. Reference sections of reviews and expert suggestions were incorporated; 29 trials were considered eligible for the meta-analysis analyzing at least one behavioral endpoint and 34 trials had complete data for BMI. Working in pairs, trained reviewers extracted study details related to the following intervention components: informational (ie, passive information, education), cognitive (ie, general cognitive strategies, goal setting, problem solving/relapse prevention), behavioral (ie, reminders and prompts for desired behaviors, skill building, practice and rehearsal, monitoring and feedback, and reinforcement of behavior), environmental (ie, physical changes made to change the environment of the school, home, and community), and parental support (ie, active involvement).

An effect size and 95% confidence interval (CI) for the difference between the intervention and control groups were calculated for each of the 4 behavioral targets (ie, increase physical activity, decrease sedentary activity, increase healthy behavior, and reduce unhealthy dietary behavior) and BMI. Standardized mean differences of about 0.2 or less were considered small, about 0.5 as moderate, and about 0.8 or greater as large effect sizes. The likelihood of between-study variability being attributable to true between-study differences (vs chance) was quantified using the I^2 statistic (inconsistency is considered small when I^2 is >25%, moderate 25%-50%, and large >50%). Several preplanned subgroup analyses of RCTs were performed.

Interventions to increase physical activity. Twenty-two randomized trials were included in the meta-analysis to assess the effects of interventions to increase physical activity. Results suggested a small increase in physical activity (effect size = 0.12; CI = 0.4 to 0.20) with moderate inconsistency across trials (I^2 = 63%) which could not be explained by subgroup analyses. There was a trend toward favoring the inclusion of multiple cognitive components (0.15; CI = 0.05 to 0.4; vs 1 or no cognitive components) and reinforcement (0.24; CI = 0.06 to 0.41; vs no reinforcement).

Interventions to decrease sedentary activity. Meta-analysis of 14 RCTs yielded a small reduction of sedentary activity (–0.29; CI = –0.35 to –0.22), with high consistency in results across studies (I^2 = 0%). Several significant treatment x subgroup interactions were detected: treatment effects were greater in trials measuring in-treatment outcomes (–0.32; CI = –0.39 to –0.25; vs outcome measured after treatment), treatment duration >6 months

(–0.31; CI = –0.39 to 0.24; vs briefer trials), and when trials involved children were enrolled (–0.31; CI = –0.39 to –0.24; vs adolescents). A trend also emerged toward favoring multiple cognitive components (–0.31; CI = –0.38 to –0.24; vs one or no cognitive components).

Interventions to increase healthy dietary behavior. Meta-analysis of 14 RCTs suggested, overall, a small and nonsignificant increase (0.06; CI = –0.09 to 0.21) with considerable heterogeneity (I^2 = 83%) in healthy dietary behavior. The trials showed great effect when reinforcement was included (0.41; CI = –0.05 to 0.76).

Interventions to reduce unhealthy dietary behavior. This category included 23 RCTs. Results indicated a small but significant reduction in unhealthy dietary behavior (–0.15; CI = –0.22 to –0.08), with greater treatment effects for trials with briefer training (–0.40; CI = –0.62 to –0.19). Thirty-four trials were examined for effects on BMI and the results were not significant (–0.02; CI = –0.06 to –0.02).

Interventions to reduce BMI. A meta-analysis of 34 RCTs of lifestyle interventions (involving 43 comparisons) on BMI failed to reveal a significant benefit (–0.02; CI = –0.06 to 0.02; I^2 = 17%). All modalities of intervention (dietary only, physical activity only, or combined lifestyle interventions) yielded similar trivial to small effects on BMI compared with controls.

Kamath CC, Vickers KS, Ehrlich A, et al. Behavioral interventions to prevent childhood obesity: A systematic review and metaanalyses of randomized trials. J Clin Endocrinol Metab.2008;93:4606-4615.

Editors' Comment: *The meta-analyses of interventions to prevent childhood obesity described above represent the first attempt to systematically quantify the benefits of cognitive, behavioral, informational, and environmental components of obesity prevention programs. While the authors acknowledged that analyses may be underpowered to detect interactions between the interventional components and the outcomes of interest; this report remains an important first step in the process of determining which intervention strategies are most effective for preventing childhood obesity. The authors discussed that previous attempts to summarize the prevention literature have been limited by the heterogeneity of the interventions and the measurement of obesity outcomes.*

Conceptually, this meta-analysis would have benefited from categorizing the prevention programs as primary (ie, all children in a given population), secondary (ie, only delivered to individuals with risk factors, such as a parent being overweight), or tertiary (ie, targeted to children who are already overweight). For the randomized trials selected for this study, categorization would be limited to primary or secondary prevention programs, as the exclusion criteria included programs with the majority of participants classified as overweight or obese. Categorizing programs in such a way would provide further information about the sample, leading us to better understand which intervention strategies may be most helpful for participants with particular characteristics and risk-profiles. It may also help us to determine if being at greater risk for obesity affects parent and child adherence to obesity prevention activities.

An additional factor to be considered when interpreting this study is the designation of intervention strategies as cognitive or behavioral. Goal-setting is considered by some to represent a behavioral strategy.[2] Generally, goal-setting is not a strategy that is used in isolation; rather, participants usually set a goal and then monitor the behavior targeted to determine how many days the goal was met. By designating goal-setting as cognitive, it may incorrectly overemphasize the importance of cognitive strategies and inadvertently diminish the impact of behavioral skills. Perhaps looking at specific strategies, rather than the classification of strategies (ie, cognitive or behavioral), may provide us with information that is easier to interpret and implement in future research studies.

We wholeheartedly agree with the authors that future publications of prevention and treatment research must detail the specifics of intervention programs. This should become a standard practice that scientific journals require.

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Brown Fat Controls – PRDM16 and Bone Morphogenetic Protein 7

Adipocytes are cells that store fats as triglycerides. White fat cells (WFC) store fats within one large, cell-filling lipid droplet. After readily available energy sources have been exhausted, the WFC hydrolyzes triglycerides and exports fatty acids to be utilized as fuel by other cells.[1] Brown fat cells (BFCs) store lipids in multiple small droplets, have a large number of mitochondria (that stain brown), and actively hydrolyze triglycerides to fatty acids which are then oxidized to produce heat. The BFC is able to oxidize fatty acids, because it expresses uncoupling protein 1 (*UCP1*, chromosome 4q31, OMIM 113730) that, in association with its co-factor (coenzyme Q),

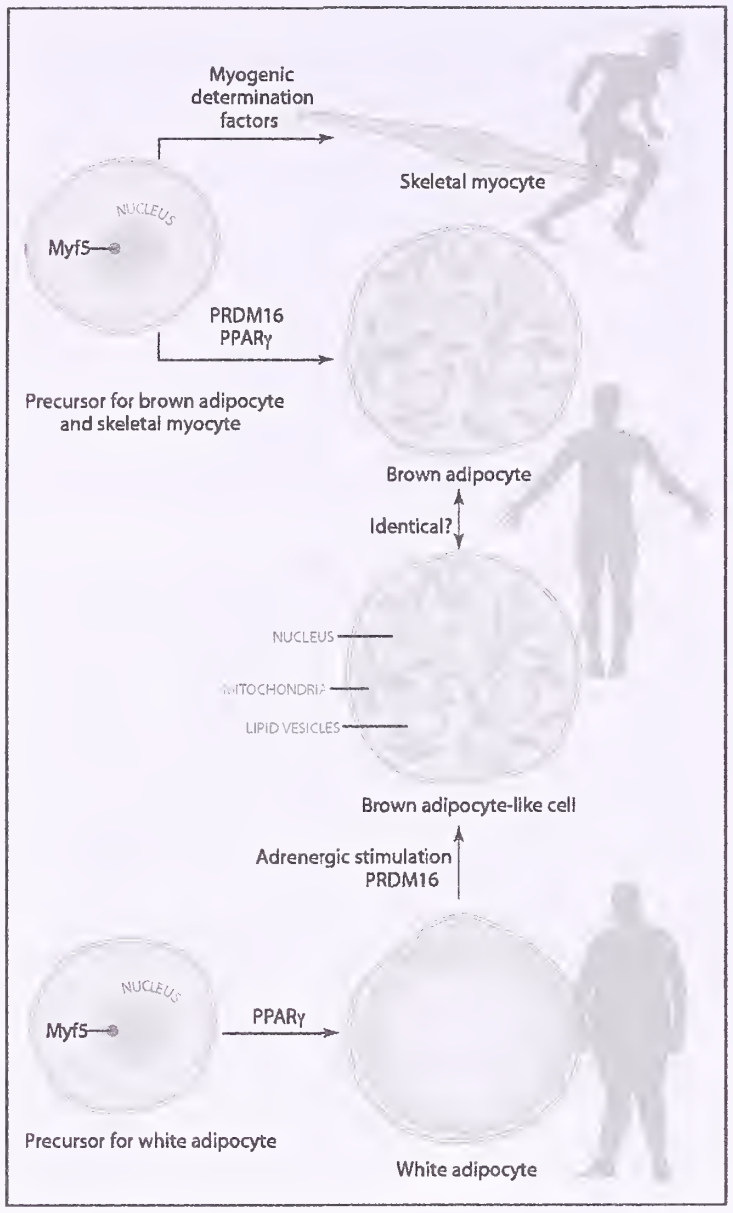

Figure 1. Paths to muscle and fat.
Skeletal myocytes and brown adipocytes derive from a common precursor cell that expresses the transcription factor Myf5. White adipocytes derive from a Myf5-negative precursor, as do brown adipocyte–like cells that appear in white fat depots after adrenergic stimulation. These distinct cell types play very different roles in physiology.

allows protons to "leak" across the inner mitochondrial membrane thereby diverting energy from ATP synthesis to (non-shivering) thermogenesis. Adipocytes are derived from a mesenchymal precursor stem cell that also gives rise to osteoblasts, chondroblasts, myoblasts, and fibroblasts. An osteoblast can be transformed to an adipocyte if *Pparγ2* (peroxisome proliferator-activated receptor γ2) is expressed, while an adipocyte can be converted to an osteoblast if *Runx2* is expressed.[2] In the presence of a β-adrenergic stimulus, a WFC can be transformed into a BFC—both morphologically and functionally. WFCs are found subcutaneously and intra-abdominally. Foci of BFCs are more abundant in infants but are also present in adults and are distinct from those BFCs that are sparsely interspersed among WFCs. It has long been assumed that the WFC and BFC arise from the same precursor cell.[3]

Searle et al now demonstrated that the BFC is actually derived from a precursor cell that differentiates into either a skeletal myocyte if it expresses a myogenic determining factor (eg, *Myf5*) or into a BFC if it expresses *Prdm16* (Proline rich domain-containing protein 16, chromosome 1p36.3, OMIM 605557) and *Pparγ2* (Figure 1). Depleting BFC precursor cells of PRDM16 in vitro resulted in their differentiation into myocytes morphologically and functionally, as these cells expressed myogenic genes rather than genes characteristic of BFCs. "Knock-in" of *Prdm16* into committed myoblasts led to their differentiation into BFCs morphologically and by expression of BFC genes. The investigators further demonstrated that the BFC that arose from the WFC in response to β-adrenergic stimulation was not derived from a myogenic precursor cell. Although there are zinc fingers within the structure of PRDM16, it does not bind to DNA but rather to other intracellular proteins. Binding of PRDM16 to PPARγ stimulates the transcriptional activity of PPARγ and BFC differentiation from the myogenic precursor cell. The authors concluded that the primary BFC is derived from a precursor cell that can differentiate either into either a myocyte or a BFC.

Tseng et al complement the findings of Searle et al by demonstrating that bone morphogenetic protein 7 (*BMP7*, chromosome 20, OMIM 112267) can also induce BFC differentiation by directing mesenchymal precursor cells to the BFC differentiation pathway. It does so by inducing expression of PRDM16 and PPARγ-coactivator-1α, a co-factor for PPARγ.

Seale P, Bjork B, Yang W, et al. PRDM16 controls a brown fat/skeletal muscle switch. Nature. 2008;454:961-967.

Tseng YH, Kokkotou E, Schulz TJ, et al. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. Nature. 2008;454:1000-1004.

First Editor's Comment: *Myocytes and BFCs arise from a common precursor cell, this links the oxidative function of these 2 cell types, and perhaps provides a reason why BFCs primarily catabolize rather than store lipids. Understanding the pathway that leads to BFC development and directed oxidation of fatty acids to thermogenesis rather that to energy generation holds the potential promise for the development of drugs (perhaps agonists of BMP7) that may be able to stimulate PRDM16 activity and BFC generation and increase dissipation of fat stores. Might agonists or antagonists of this pathway even be of use in clinical conditions in which control of core body temperature is indicated?*

Allen W. Root, MD

Second Editor's Comment: *A recent paper published in the New England Journal of Medicine highlighted*

the cold-activated brown adipose tissue in healthy men.[4] An accompanying editorial highlighted the pathophysiology of this tissue and the potential implication for stimulating energy expenditure (Figure 2).[5] These 2 papers proposed the concept of target interventions—pharmacological and environmental—aimed at modulating energy metabolism. Wouldn't it be great if lowering the thermostat could help prevent and treat obesity?

Fima Lifshitz, MD

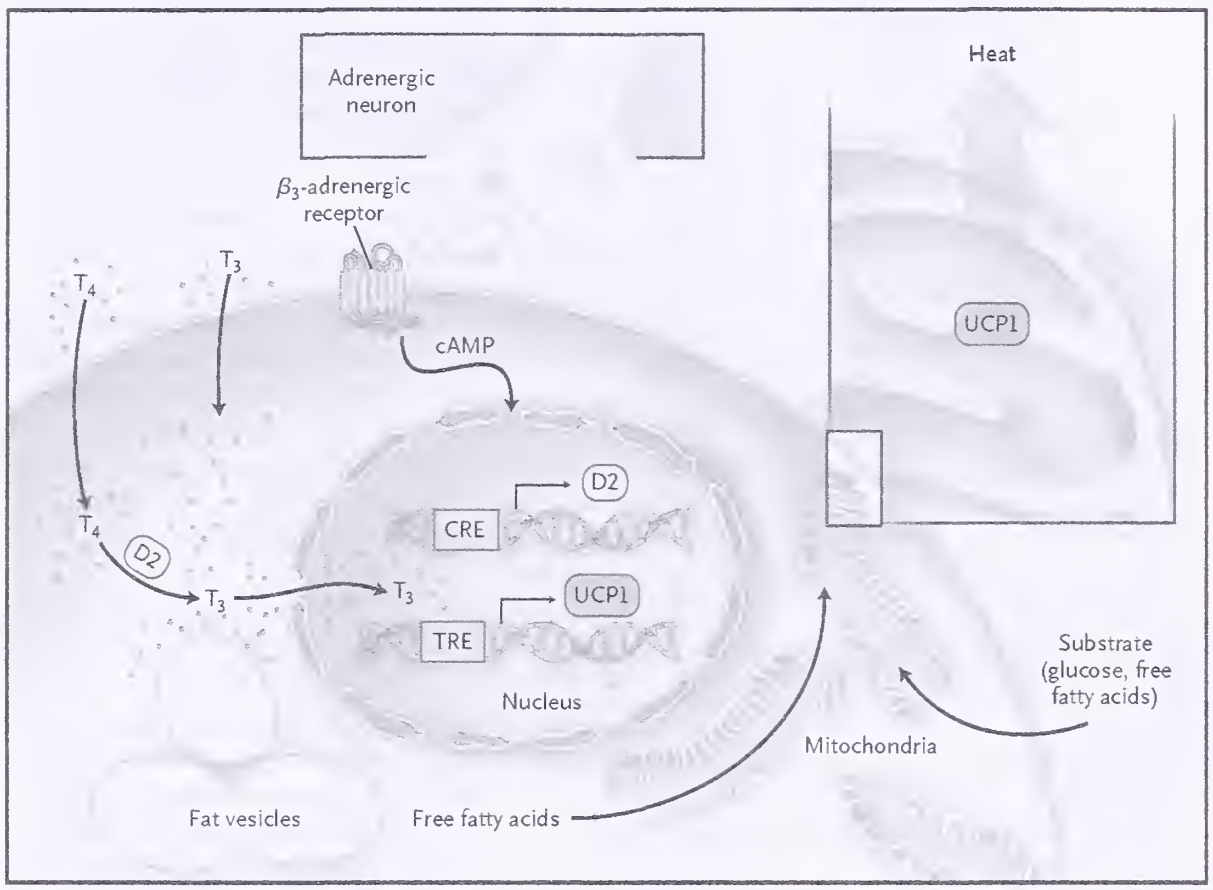

Figure 2. The Activation of Brown Adipose Tissue.
Stimulation of β_3-adrenergic receptors leads to the dramatic increase in the intracellular concentration of triiodothyronine (T_3) by means of the type 2 5′ deiodinase (D2); T_3 in turn stimulates the transcription of uncoupling protein 1 (UCP1), which causes the leakage of protons from the inner membrane of the mitochondria, hence dissipating energy in the form of heat. The abbreviation cAMP denotes cyclic adenosine monophosphate, CRE cAMP response element, T_4 thyroxine, and TRE thyroid hormone response element.

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Reciprocal Regulation of Bone and Energy Metabolism

Remodeling allows bones to renew themselves and this process requires a fair amount of energy. In view of this, Lee and Karsenty hypothesized that there is somewhat of a common control of bone and energy metabolism. That started a search for a bone derived hormone that in turn regulates energy metabolism. The clinical observation that obesity protects from osteoporosis led to the proposal that bone remodeling was dependent, in some way, from an adipose tissue derived hormone, leptin. The researchers showed that leptin regulates bone mass. It binds to its receptor on hypothalamic neurons and then uses 2 pathways: (1) sympathetic signaling in osteoblasts which favors osteoclast differentiation by inducing *RANKL* gene expression, and (2) through CART (cocaine-and amphetamine-regulated transcript) inhibiting the *RANKL* expression in osteoblast (Figure 1). In view of these data they raised the question: is the skeleton, in turn, regulating any aspect of energy metabolism? This led to the search of a bone derived hormone.

In search of that hormone, an osteocalcin$^{-/-}$ mouse was generated. It displayed a high bone mass phenotype and it also had an abnormal amount of visceral fat. This was the first evidence suggesting that skeleton regulates energy metabolism. Thereafter, mutant mice-lacking genes, expressed preferentially in osteoblast, were generated. The first gene of interest was *Esp*; it encodes a receptor like protein tyrosine phosphatase termed OST-PTP4. These mice had a surprising phenotype pointing towards new modes of regulation of glucose metabolism: increased insulin secretion with hypoglycemia, increase in beta cell proliferation, and an increase in insulin sensitivity. Given the increase in insulin sensitivity, the mutants should be fatter. Instead they had

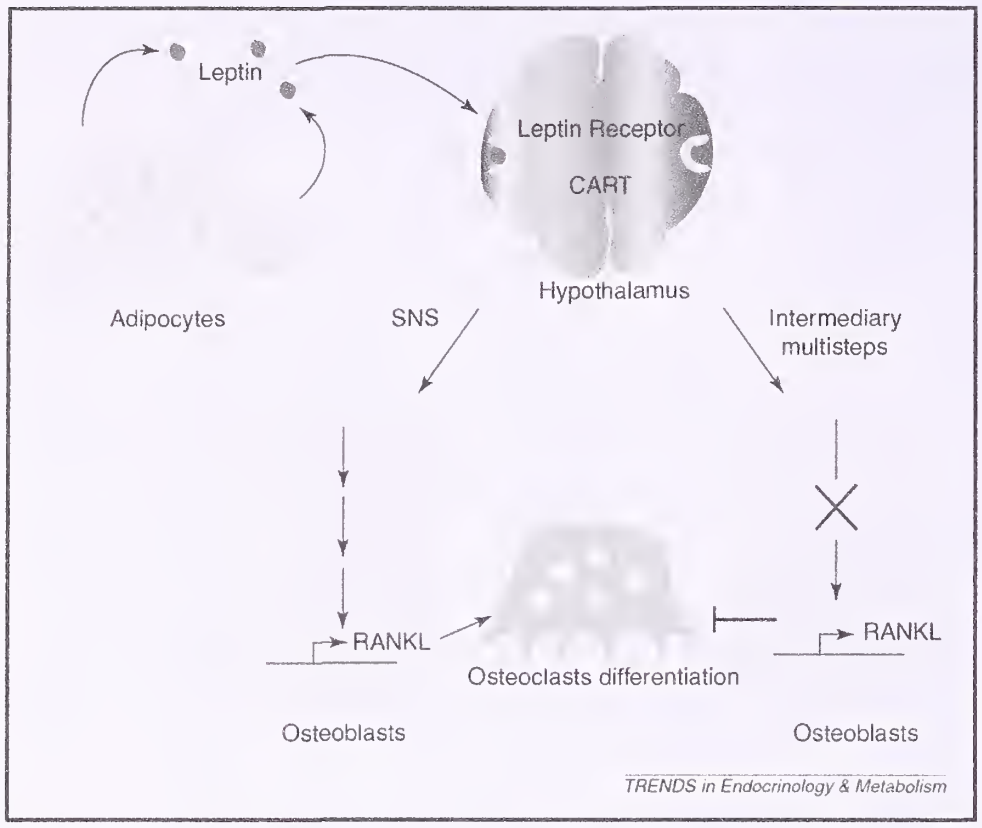

Figure 1. Schematic representation of bone mass regulation by fat. The adipocyte-derived hormone leptin binds to its receptor on hypothalamic neurons and then uses the sympathetic tone and cocaine- and amphetamine-regulated transcript (CART) to regulate bone function and RANKL expression in osteoblasts.
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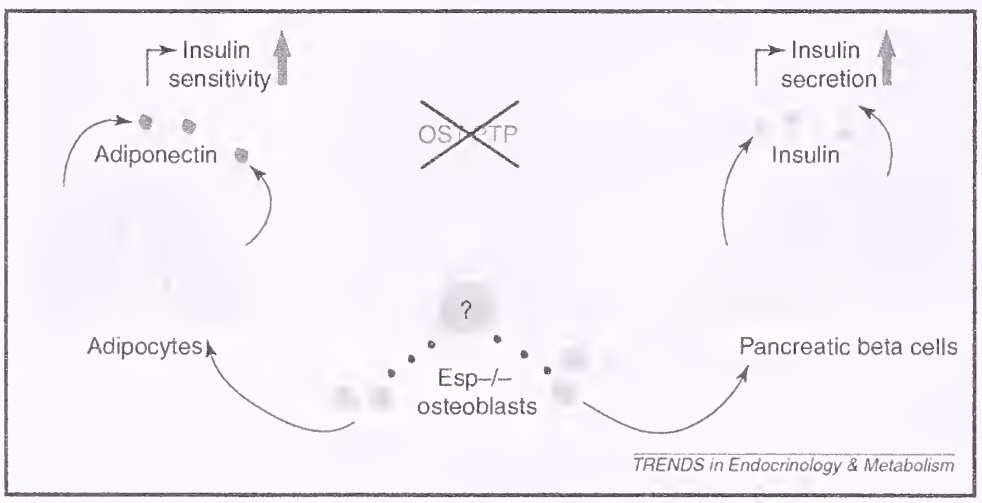

Figure 2. Osteocalcin in its uncarboxylated form is an osteoblast-derived hormone that improves glucose handling. OST-PTP, the Esp gene product, favors, through yet unknown mechanisms, the carboxylation of osteocalcin. In the absence of Esp, more osteocalcin is uncarboxylated. This uncarboxylated form of osteocalcin increases expression of the insulin gene in b cells and the expression of the adiponectin gene in adipocytes, resulting in an increase in insulin secretion and in insulin sensitivity, respectively.
Reprinted with permission Lee NK, Karsenty G. Trend Endocrinol Metab. 2008;19:161-166.

less fat because of an increase in energy expenditure; furthermore appetite was not affected. In addition, mice overexpressing *Esp* only in osteoblasts developed type 2 diabetes phenotype on a normal diet.

Osteocalcin was shown to be the molecule made by osteoblasts that accounts for the osteoblast-mediated regulation of gene expression of insulin in islets and of adiponectin, an insulin-sensitizing adipokine in adipocytes. It then appears that the *Esp*-deficient mice metabolic phenotype is caused by a gain of osteocalcin bioactivity and that OST-PTP regulates indirectly osteocalcin's post-translational modifications; it favors the carboxylation of osteocalcin to transform it into bone gla protein. A small portion of non carboxylated osteocalcin is circulating and has the ability to improve glucose handling as described (Figure 2).

The authors further evaluated the potential relevance of these findings. They interpreted a series of experiments concluding that the increase in insulin sensitivity might protect from diabetes in a situation in which insulin secretion is low but not absent.

Lee NK, Karsently G. Reciprocal regulation of bone and energy metabolism. Trend Endocrinol Metab. 2008;19:161-166.

Editor's Comment: *In recent years this provocative work has attracted great attention. It shows that communication from metabolism to bone is not unidirectional and that bone regulates glucose metabolism and fat mass via the uncarboxylated form of osteocalcin in a complex crosstalk.[1] Therefore it is of interest that the association between serum osteocalcin concentration and markers of dysmetabolic phenotype was evaluated in a group of adults.[2] It was shown that serum osteocalcin was inversely associated with blood markers of dysmetabolic condition and measures of obesity. These findings need to be replicated to further test the initial hypothesis and to be extended to further studies in relation to type 2 diabetes. There is presently a major interest in this new presentation of energy metabolism including bone as a major actor.*

Raphaël Rappaport, MD

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Brain-Derived Neurotrophic Factors and Obesity in WAGR Syndrome

The syndrome of Wilms' tumor, aniridia, anomalies of the genitourinary tract including ambiguous external genitalia, mental retardation and hemihypertrophy (*WAGR* - OMIM 194072, chromosome 11p13, Figures 1 and 2) is associated with heterozygous microdeletions of chromosome 11p13 and loss of the contiguous genes *WT1* (OMIM 607102) and *PAX6* (OMIM 607108). Obesity has been found in some subjects with this disorder. Also on chromosome 11p13 is the neuronal survival factor, brain-derived neurotrophic factor (*BDNF*, OMIM 113505), which has been found to affect energy metabolism in rodents. Loss of *Bdnf* in mice results in excessive weight gain in adulthood due to increased caloric intake.[1] Thus, BDNF may be an anorexigenic factor. In order to examine the effect of BDNF on energy metabolism in humans, the investigators examined the relationship between the presence or absence of *BDNF* and weight in patients with WAGR by examining the extent of the deletion at chromosome 11p13 in 33 patients. Haploinsufficiency of *BDNF* was present in 19 patients–complete in 17 and partial in 2 subjects. By 2 years of age, weight was greater in all WAGR patients with loss of *BDNF* than in those with an intact gene. Serum concentrations of BDNF were

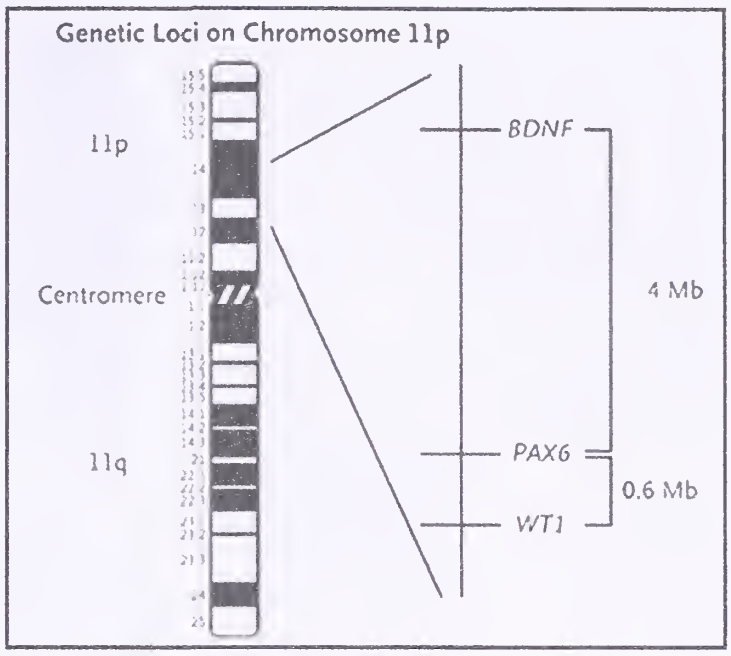

Figure 1. Genetic Loci on Chromosome 11 in Patients with the WAGR Syndrome.
The WAGR syndrome is caused by deletions on chromosome 11p that result in haploinsufficiency for the *PAX6* and *WT1* genes. *BDNF* is located approximately 4 Mb telomeric to *PAX6*.
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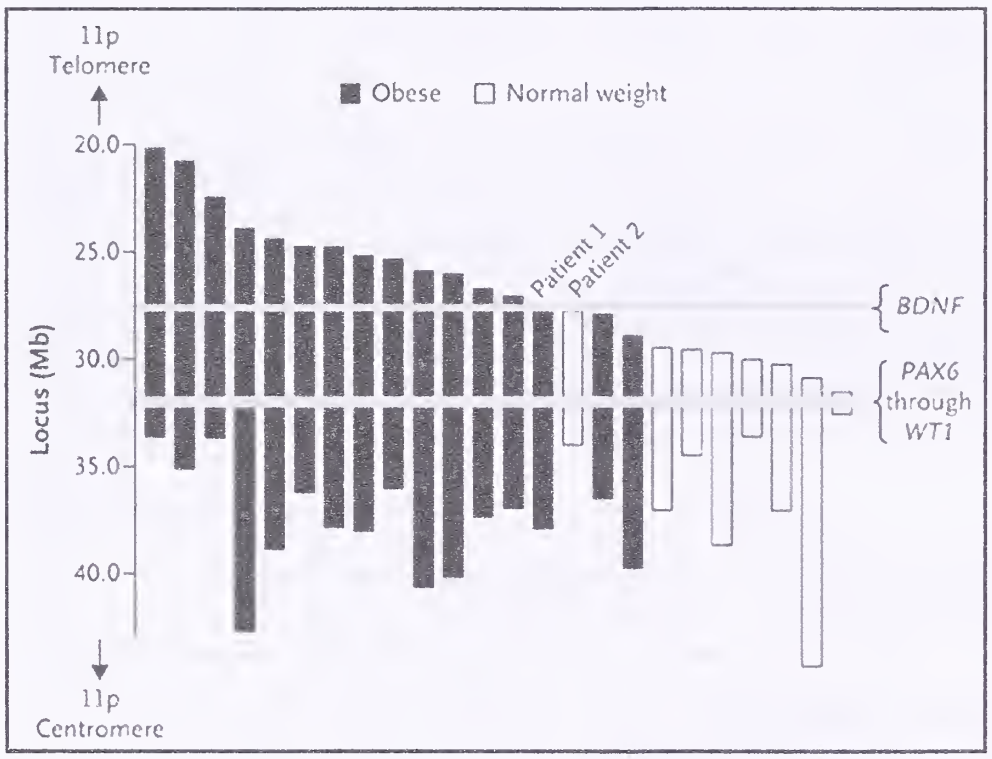

Figure 2. Regions of Deletion on Chromosome 11p.
The region of deletion on chromosome 11p is shown for each of the 24 patients in whom the presence or absence of childhood obesity (body-mass index [BMI] ≥95th percentile by 10 years of age) could be determined. No association between the centromeric deletion boundary and childhood obesity was observed. However, for the telomeric deletion boundary, all patients with heterozygous deletion of all or a portion of BDNF had childhood obesity, whereas no deletions involved BDNF in the patients who were of normal weight. In Patient 1, who was obese, there was a heterozygous deletion of BDNF exons 1 through 3. In Patient 2, who had a normal weight (BMI, approximately 20th percentile at 10 years of age), the deletion region ended 72.5 kb upstream of BDNF. Only 20% of the patients without BDNF deletions were obese; this rate is similar to the prevalence of childhood obesity in the general U.S. population.[6]
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higher and hyperphagia was more prevalent in the former subjects. Loss of any exon of *BDNF* or of a portion of the 70 to 80-kb region upstream of *BDNF* was associated with obesity. The investigators also demonstrated that heterozygous loss of *BDNF* was associated with decreased pain perception in WAGR subjects. The authors concluded that BDNF modulates appetite in humans as well as in experimental animals.

Han JC, Liu QR, Jones MP, et al. Brain-derived neurotrophic factor and obesity in the WAGR syndrome. N Engl J Med. 2008;359:918-927.

Editor's Comment: *These observations suggest that there well may be patients with childhood onset obesity and loss-of-function mutations in BDNF itself or in its 5' region and/or that a polymorphic variant(s) of this gene may be related to appetite, caloric intake, and body weight. In the hierarchal mechanisms that regulate appetite, BDNF may function downstream of the melanocortin 4 receptor and thus be responsive to the effects of αMSH and proopiomelanocortin.*[2] *Other clinical studies have demonstrated a strong association between the*

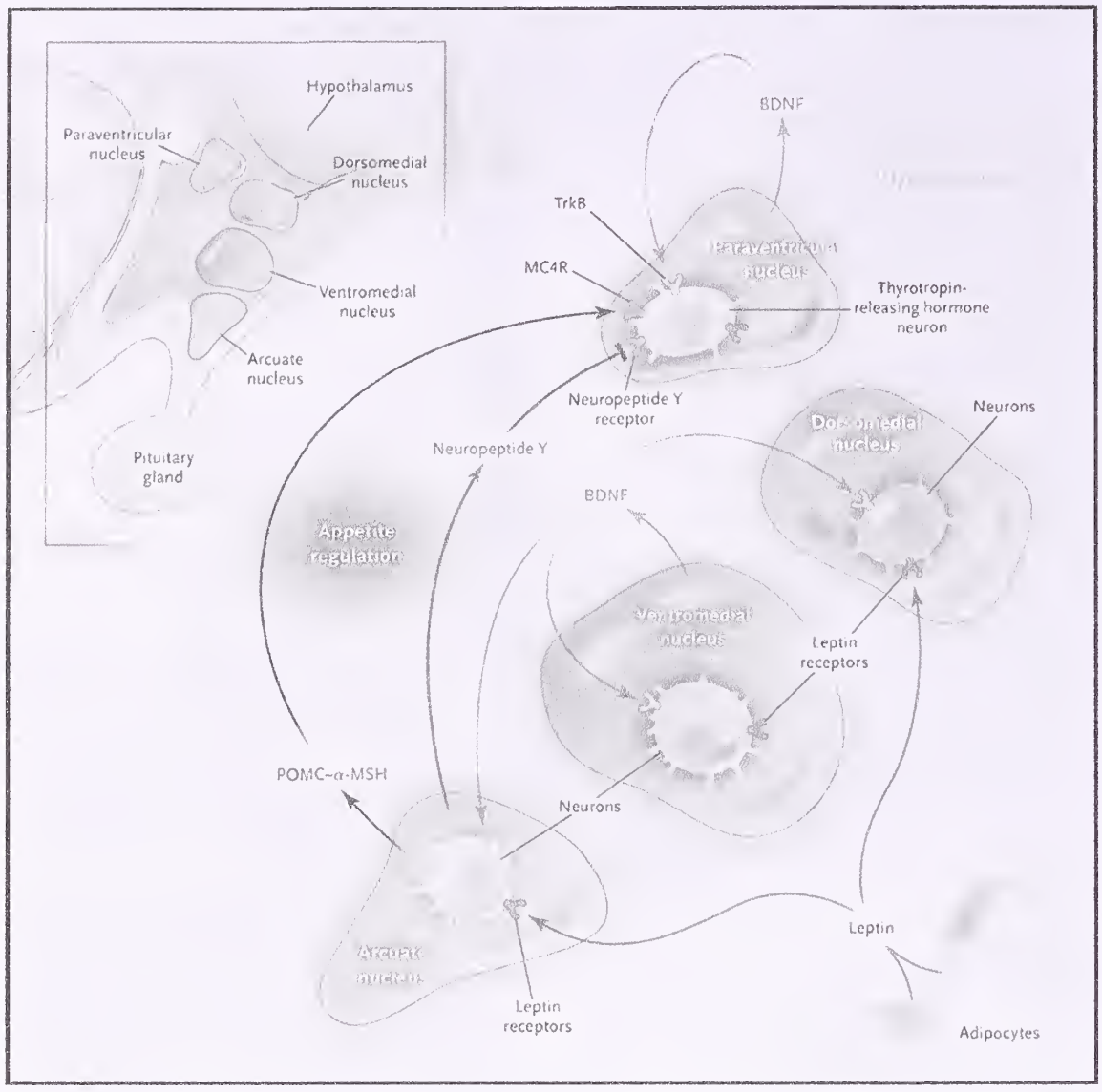

Figure 3. Model of the Anorectic Action of Brain-Derived Neurotrophic Factor (BDNF) in the Hypothalamus. BDNF is produced by thyrotropin-releasing hormone neurons in the paraventricular nucleus and acts on neurotrophic tyrosine kinase receptor type 2 (TrkB). BDNF-producing neurons are excited by the release of α-melanocyte–stimulating hormone (α-MSH) from proopiomelanocortin (POMC) neurons in the arcuate nucleus, which acts on melanocortin 4 receptors (MC4Rs). BDNF-producing neurons in the paraventricular nucleus are inhibited by neuropeptide Y that is released from the arcuate nucleus. Leptin from the periphery can also excite BDNF-producing neurons that express the signaling form of the leptin receptor. Adapted from Levin.[7]

BDNF Met66 allele of the Val66Met polymorphic variant and anorexia nervosa and other eating disorders.[3] Interestingly, the Val66 allele of BDNF has been associated with child onset bipolar disorder.[4] The model of the anorectic action of BNDF in the hypothalamus[5] is shown in Figure 3.

Allen W. Root, MD

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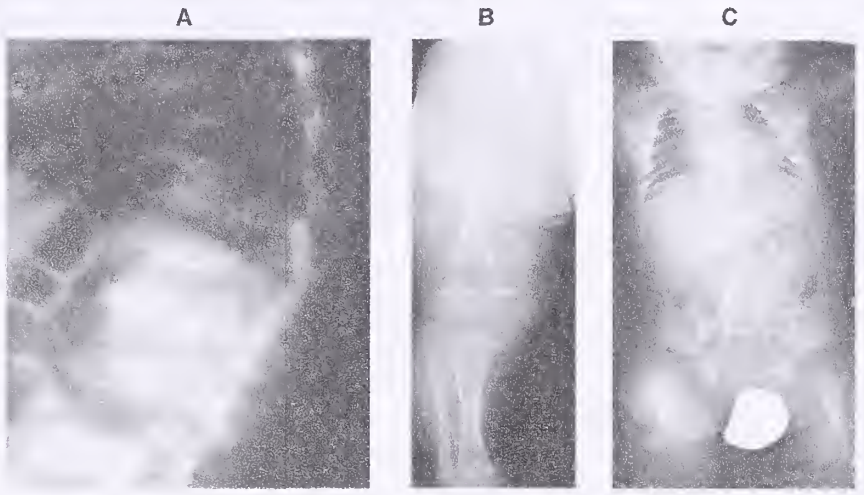
Calcium Channel Family of Bone Dysplasias

Brachyolmia is a relatively mild bone dysplasia that primarily affects vertebral body growth leading to mild to moderate short trunk dwarfism. One form of autosomal dominant brachyolmia (MIM 113500) was recently shown to result from activating mutations of TRPV4, a calcium-permeable ion channel protein that has been implicated in skeletal development.[1] Qualitatively similar but more severe radiographic changes are found in 2 other dominantly inherited bone dysplasias. The first, spondylometaphyseal dysplasia Kozlowski type (SMDK, MIM 1842522), is characterized by postnatal onset of short stature, kyphoscoliosis and progressive deformity. The second, metatropic dysplasia (MIM 156530), presents in newborn infants with short limbs, but evolves to a short trunk clinical phenotype as a result of severe and progressive kyphoscoliosis typically compromising neurologic and respiratory functions. The clinical and radiographic similarities prompted Krakow and colleagues to search for mutations of TRPV4 in the latter disorders, which



Typical radiographic manifestations of SMDK (A,B) and metatropic dysplasia (C). Modified from Krakow D, et al. Am J Hum Genet. 2009;84:307-315. Copyright © Elsevier 2009. All rights reserved.

they have now reported.

Heterozygous missense mutations TRPV4 were detected in all 8 patients who were studied, 6 with SMDK and 2 with metatropic dysplasia. One mutation was recurrent in 4 patients with SMDK. It and 2 other mutations mapped to the cytoplasmic domain of the channel protein where the brachyolmia mutations had mapped, but 2 mapped to so-called ankyrin repeats, a common molecular motif thought to be involved in folding and direct interactions between proteins.

Since gain of channel function had been implicated in the brachyolmia-associated mutations of TRPV4, the investigators analyzed basal channel activity and responses to known TRPV4 agonists and antagonists of the 4 SMDK-associated mutations. Three displayed increased basal channel activity. The responses to agonists and antagonists were less clear and the authors concluded that the mutations most likely act through increasing basal intracellular calcium. They also noted that genetically engineered mice lacking TRPV4 function exhibit a defect in osteoclast function associated with overmineralized bone and suggest that the clinical phenotype in brachyolmia, SMDK and metatropic dysplasia may reflect disturbed TRPV4 function in both chondrocytes and osteoblasts during bone growth.

Krakow D, Vriens J, Camacho N, et al. Mutations in the gene encoding the calcium-permeable ion channel TRPV4 produce spondylometaphyseal dysplasia, Kozlowski type and metatropic dysplasia. Am J Hum Genet. 2009;84: 307-315.

Editor's Comment: *The family concept that disorders that exhibit qualitatively similar clinical phenotypes result from mutations of the same gene function continues to be borne out, in this case with TRPV4. It will be interesting to watch this story unfold regarding how abnormal calcium channel activity or increased intracellular calcium, as the authors proposed, alters the biology of bone growth. There are a number of drugs used to treat various diseases unrelated to bone growth and there are health food constituents that are thought to affect intracellular calcium concentrations. One wonders if these agents could counter the adverse effects of disturbed TRPV4 channel function in cells that contribute to bone growth.*

William A. Horton, MD

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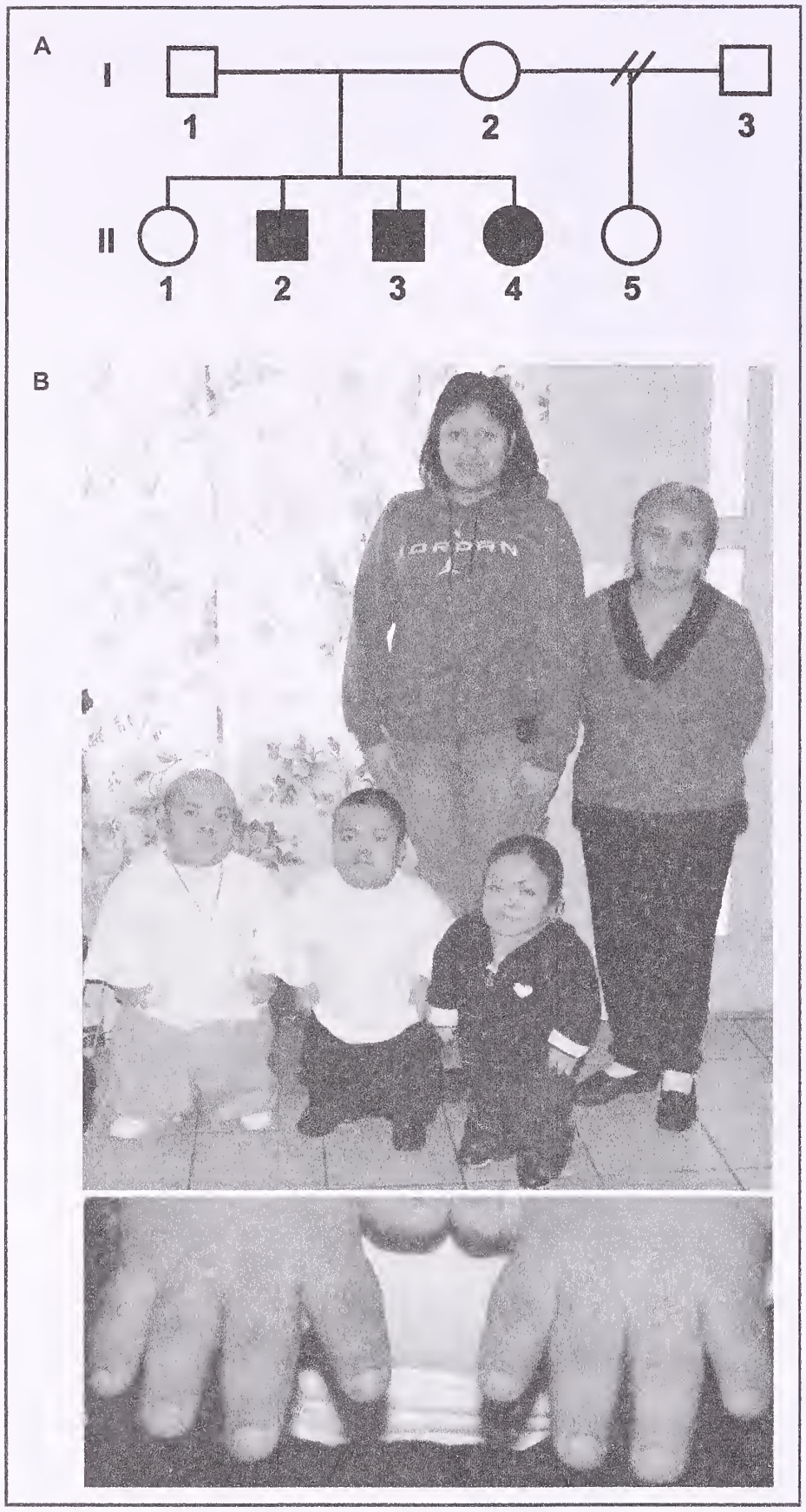
Spondyloepimetaphyseal Dysplasia – Aggrecan

Despite considerable progress in delineating the human chondrodysplasias, there are still many distinctive dwarfing clinical phenotypes for which no mutant gene has been found; conversely, there are genes that encode proteins important for linear bone growth for which few if any chondrodysplasia-causing mutations have been identified. The report by Tompson et al helps to establish a new link in this context.

A family is described with extreme short stature, brachydactyly, distinctive facies and radiographs consistent with spondyloepimetaphyseal dysplasia (SEMD) in 3 of 4 siblings; the parents had average stature suggesting autosomal recessive inheritance (Figure). Consanguinity was denied, but both parents came from a small village in Mexico. Whole-genome single-nucleotide polymorphism (SNP) analysis of the 2 affected siblings and 1 unaffected sibling revealed several 10-20 cM blocks of shared alleles suggesting a common ancestry and raising the possibility that the affected siblings may have inherited an ancestral mutation that was transmitted through both parents. If so, then they would be expected to be homozygous for the mutation, ie, identity by descent, and the mutation would be expected to map to the genetic region or interval that was shared by the affected siblings but not by the unaffected sibling. A single genetic interval on chromosome 15 met these criteria; it contained 193 annotated or characterized genes and 103 unannotated genes.

When the investigators narrowed down their search to genes expressed only or disproportionately highly in cartilage, because of its essential role in endochondral bone growth, 2 genes emerged: chondroitin sulfate



Clinical Phenotype. In the top image, the back row from left to right shows II-1 (23 years old [yo]) and I-I (58 yo), respectively, and the front row from left to right shows II-2 (19 yo), II-3 (16 yo), and II-4 (26 yo), respectively. Note the telescoping fingers of II-3.
Modified from Tompson SW, et al. A J Hum Genet. 2009;84:72-79. Copyright © Elsevier 2009. All rights reserved.

proteoglycan 4 (CSPG4) and aggrecan (AGAN). Sequence analysis of AGAN revealed homozygosity in the affected siblings for a missense mutation that substituted an asparagine for an aspartic acid residue at position 2267 (A2267N) in the C-terminal G3 globular domain of aggrecan. The parents were both heterozygotes for the mutation.

The A2267 aspartic acid is highly conserved across species and even across different proteoglycans that share a similar globular domain; it has been implicated in mediating molecular interactions between aggrecan and other cartilage matrix constituents, such as tenascin. To explore the molecular consequences of the mutation, the authors expressed the normal and mutant G3 domain proteins in cells and then analyzed them biochemically. They observed that the mutant G3 domain was secreted normally, but there was evidence of disturbed binding of the mutant G3 domain to tenascin compared to normal. They also showed that the asparagine residue in the mutant AGAN G3 domain is capable of being glycosylated, which could potentially alter functions as well as biosynthesis and stability of AGAN.

The authors noted that haploinsufficiency due to heterozygous mutations of AGAN has recently been reported in another rare condition, spondyloepiphyseal dysplasia (SED) Kimberley. This is a milder condition that typically presents with mild short stature and precocious osteoarthritis. They also point out that the genomic region to which AGAN has been mapped through genome-wide association studies has been linked to normal height variation.

Tompson SW, Merriman N, Funari V, et al. A recessive skeletal dysplasia, SEMD aggrecan type results from a missense mutation affecting the C-type lectin domain of aggrecan. A J Hum Genet. 2009;84:72-79.

Editor's Comment: *Given its abundance in growth plate cartilage and presumed importance to endochondral bone growth, the difficulty finding mutations of AGAN in chondrodysplasias is surprising. It would have been interesting to study cartilage tissue, which presumably was not available, since it might have provided additional clues as to how the mutation disrupts cartilage biology. A factor not discussed is the possibility that alterations in cartilage aggrecan*

could alter growth factor signaling within the growth plate, as proteoglycans are thought to influence the mobility and local concentrations of growth factors in cartilage and possibility their presentation to transmembrane receptors.

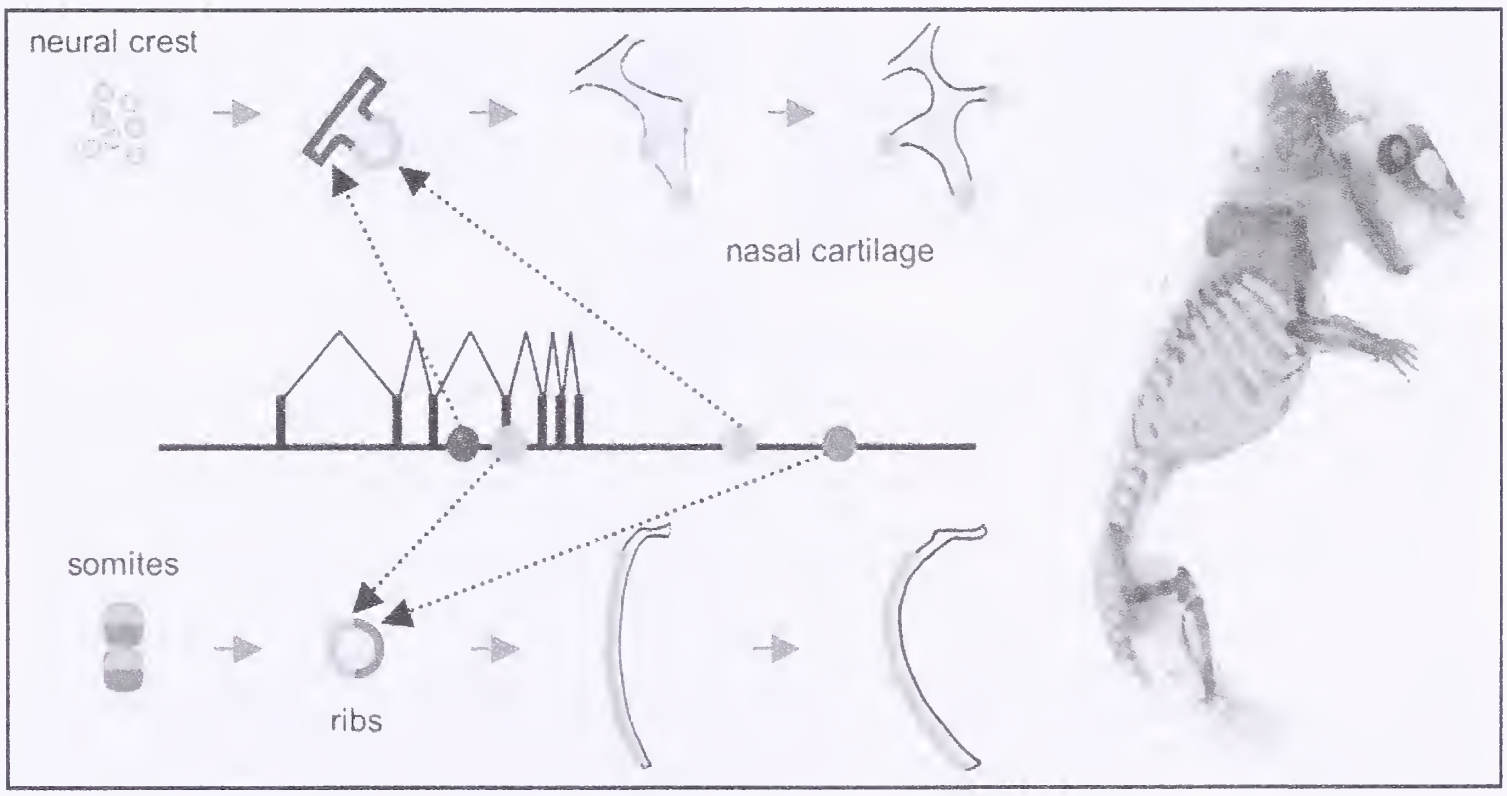
William A. Horton, MD

Anatomy-Specific Enhancers of BMP Genes Fine-Tune Size and Shape of Individual Bones

As the skeleton grows, constituent cartilage and bone tissues are formed into a remarkable range of sizes and shapes. Although the blueprints that sculpt individual bones must be encoded in the genome, little is known about how this occurs. A group headed by David Kingsley at Stanford has recently provided novel insight into how the anatomy of individual bones is regulated. Their story begins with the generally accepted concept that bone shapes are determined by differential growth and erosion along the surfaces of bones. For instance, preferential deposition and erosion on opposite surfaces of a bone would generate lateral displacement or curvature of the bone such as a rib. Localized regions of deposition and erosion would shape ridges, foramina, and other surface structures.

The group focused their attention on the BMP5 gene because it is surrounded by large genomic regions containing regulatory elements required for normal developmental regulation and on rib development because BMP5 is expressed in the perichondrium surrounding ribs and ribs are suitable for detecting differential growth and erosion. The approach was to generate transgenic mouse embryos harboring both a *lacZ* reporter gene and genomic DNA corresponding to different regions of the BMP5 locus including surrounding genomic DNA. ß-galactosidase staining of late-stage transgenic embryos revealed specifically where the regulatory regions, ie, presumed enhancers, were active.

The details of the experiments are beyond the scope of this abstract. However, a regulatory element within the coding region of the gene was found to drive expression of BMP5 in the perichondrium adjacent to the lateral aspect of the ribs, whereas regulatory sequences 100 kb 3' to the coding region drove expression in the perichondrium of the medial aspect of the ribs. A number of confirmatory experiments was done, all of which suggested that BMP5 expression in different domains of the rib perichondrium is controlled by distinct regulatory elements in or near the BMP5 locus. In other words, anatomy-specific enhancers in BMP genes may provide



Discrete enhancers control growth in distinct anatomical domains of developing bones. Multiple anatomy-specific enhancers (filled circles) are spread across the Bmp5 locus. In ribs, 2 enhancers (green and purple circles) may respond to lineage domains established in somites to control growth on opposing sides of the ribs. Local growth on the lateral edge of rib surfaces promotes rib curvature and expansion of the thoracic cavity. Nasal cartilages form from cranial neural crest. Two enhancers (blue and orange circles) in the Bmp5 gene are expressed in different highly restricted locations, leading to characteristic branching patterns of the nasal turbinates.

a genomic mechanism for independent developmental control of local growth of individual bones.

During these studies, the investigators also discovered 2 regulatory elements that controlled expression of BMP5 in nasal cartilage. These elements were distinct from those controlling BMP expression in ribs but like them mapped to locations within and 3' from the coding region of the gene as shown in the Figure. The authors suggested that the proposed mechanism may not be limited to regulation of BMP5 but common to other developmentally regulated genes that are involved in fine-tuning morphogenesis.

Guenther C, Pathalena-Filho L, Kingsley DM. Shaping skeletal growth by modular regulatory elements in the Bmp5 gene. PLoS Genetics. 2008;4:1-13.

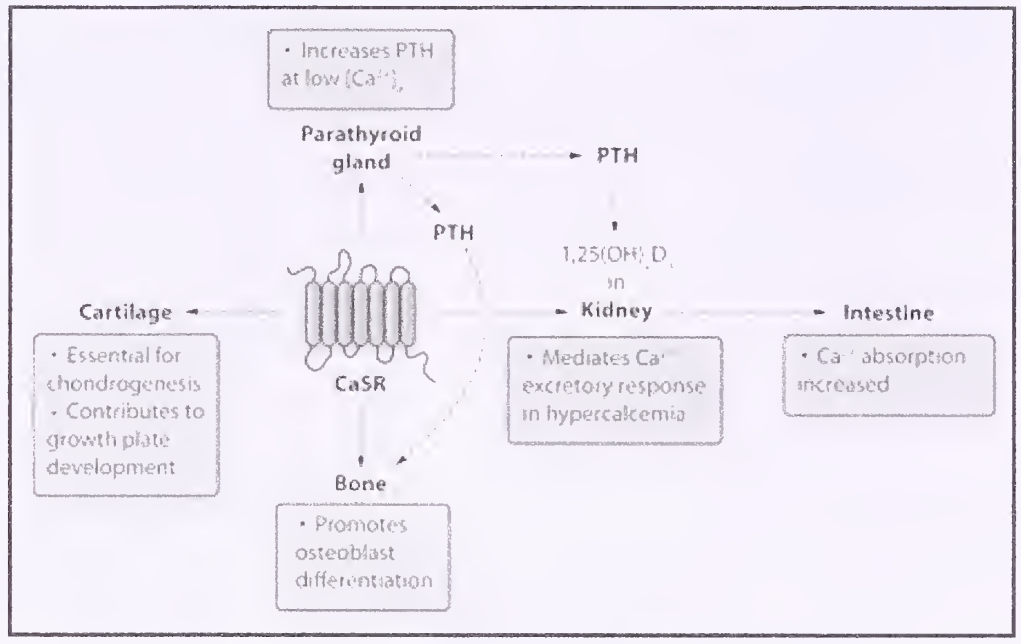
Editor's Comment: *This investigation provides novel insight into the fine-tuning of skeletal development. It is interesting to speculate how subtle radiographic findings that allow experts to distinguish between similar bone dysplasias might reflect disturbances in these regulatory mechanisms.*

William A. Horton, MD

Extracellular Calcium-Sensing Receptor: Modulator of Skeletal Development

The roles of the calcium-sensing receptor (CaSR) as a negative regulator of parathyroid hormone (PTH) synthesis and secretion and as an inhibitor of calcium reabsorption by the renal tubule are well documented; its functions in chondrocytes, osteoblasts, and osteoclasts have been less completely documented. In part, this has been due to inability to generate mice in which *Casr* has been ablated specifically in cartilage and bone cells and to the expression in these cells of an alternatively spliced, functionally active isoform of the CaSR.[1] *Casr* is expressed in differentiating osteoclasts, chondrocytes, and osteoblasts. Generalized ablation of *Casr* in mice results in a rachitic phenotype (increased width of the zone of hypertrophic chondrocytes, depressed and disordered calcification of the cartilage growth plate, and decreased rate of cartilage mineralization).[2]

The present investigators have developed strains of mice in which exon 7 of *Casr* (encoding the 7 transmembrane and 4 intracellular loops of the receptor protein) has been specifically "knocked-out" in parathyroid cells (PTC), growth plate chondrocytes (GPC), and osteoblasts (OB) rendering the *Casr* functionally inactive. Homozygous loss of *Casr* in PTC resulted in impaired growth and death within 2 weeks after birth. As anticipated, these mice had increased expression of *Pth* in their PTC. The skeletons of these mice had abundant matrix but were markedly undermineralized, and multiple fractures were present. There was substantially decreased expression of *Casr* in bone cells and delayed OB differentiation. In mice in which *Casr* was specifically ablated in OB, the phenotype of growth retardation, skeletal undermineralization with increased osteoid formation, multiple fractures, and death by 3 weeks of age was observed. OB differentiation was severely impaired as was OB expression of *Igf1*. The rate of apoptosis of OBs was accelerated. The homozygous loss



Classic Ca^{2+} homeostasis (dashed arrows) and novel developmental functions (arrows) of CaSR have been revealed by cell type–specific null mutations of *Casr* in the mouse. CaSR is found in bone, kidney, and gut, which are the three main Ca^{2+}-mobilizing organs. The normal homeostatic signaling pathways between these organs and the parathyroid gland have been detailed previously.[5] The functions performed by CaSR in each organ are outlined in boxes. To maintain normal Ca^{2+} homeostasis, CaSR in parathyroid cells (PTCs) senses alterations in $[Ca^{2+}]e$. The release of parathyroid hormone (PTH) enables bone and kidney to respond in a manner to normalize $[Ca^{2+}]e$, through the activation of key responses in kidney [production of $1,25(OH)_2D_3$ and reabsorption of Ca^{2+}], intestine [Ca^{2+} absorption through the increased abundance of $1,25(OH)_2D_3$], and bone matrix resorption through PTH (not shown). The direct role of CaSR in the intestine is questionable because an intestine-specific knockout of Casr has not been performed. Targeted knockout of *Casr* through the crossing of *Casr* floxed mice with mice expressing *Cre* under the control of tissue-specific promoters has identified novel functions for CaSR in skeletal development. Ablation of *Casr* in the parathyroid gland resulted in the expected phenotypes that occur in patients with inactivating mutations in *Casr*, such as hyperparathyroidism and hypercalcemia. Deletion of Casr in chondrocytes demonstrated a requirement for CaSR in early skeletal development, whereas a role for CaSR in promoting bone cell differentiation was determined by deletion of *Casr* in cells of the osteoblast lineage.

of expression of *Casr* in GPC was lethal; affected embryos died before day 13 of embryonic life. Development of a mouse model in which "knock-down" of *Casr* expression in GPC at day 16 to 17 of embryonic life after treatment with an estrogen receptor agonist (tamoxifen) resulted in offspring with modestly decreased growth of long bones despite expansion of the hypertrophic zone of the growth plate, decreased differentiation to terminal chondrocytes, and decreased expression of *Igf1* and *Igf1r* by GPC.

The authors concluded that: (1) the elimination of a functional CaSR in PTC also depressed *Casr* expression in osteoblasts (hypothetically through hypercalcemia and increased signaling by the PTH receptor in bone); (2) the CaSR was innately essential for osteoblast differentiation, function, and survival; and (3) that partial and delayed loss of the CaSR in hypertrophic chondrocytes reduced chondrocyte differentiation in part through decreased IGF-1R signaling.

Chang W, Tu C, Chen TH, Bikle D, Shoback D. The extracellular calcium-sensing receptor (CaSR) is a critical modulator of skeletal development. Sci Signa. 2008;I: ra1\. [DOI:10.1126/scisignal.1159945]

Editor's Comment: *This research has demonstrated the individual importance of the CaSR in PTCs, OBs, and hypertrophic chondrocytes.[3] Interestingly, "knock-out" of the CaSR in PTCs secondarily impaired expression of Casr in osteoblasts, demonstrating clearly the interdependence of the parathyroid-osteoblast axis. A study of the effect of overexpression of Casr in PTCs upon OB expression of Casr and bone morphology would be of interest. This manuscript also introduced a new feature of the electronic journal—Science Signaling—sponsored by the AAAS, that has until now published review articles and didactic materials on the subjects of intra- and intercellular communications.[4] It will now publish original research articles as well.*

Allen W. Root, MD

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Insulin Analogues...and Cancer?

Insulin analogues with different pharmacokinetics were created by inserting point modifications into the amino acid sequence of human insulin, particularly the C-terminus of its beta-chain which is not involved in binding to the insulin receptor (IR). However, these modifications may alter binding affinity for the closely related type 1 insulin-like growth factor (IGF) receptor (IGF1R). Thus, Weinstein et al asked the important question of how these analogues compare to insulin and IGF-I in eliciting IGF-I activities (namely, proliferation and protection from serum starvation-induced apoptosis) in cultured cancer cells.

They studied 2 long-acting insulin analogues (glargine [Lantus®] and detemir [Levemir®]) and 2 short-acting analogues (lispro [Humalog®] and aspart [Novolog®]) in 3 different cell lines: HCT-116 colorectal, PC-3 prostate and MCF-7 breast cancer cells. All experiments were conducted in vitro. Results are summarized in the Table.

HCT-116 cells showed a dose-dependent proliferative response to both glargine and detemir at 72 hours, but not IGF-I (all doses about +21%) nor insulin (all doses negligible effect). The authors then turned to signaling pathways that may underlie the hormonal effects in HCT-116 cells. Basal expression levels of IR and IGF1R were equivalent when measured by Western immunoblotting and immunofluorescent staining. After

Summary of effects on cell behavior in vitro.

Effect	Insulin	Glargine	Detemir	Lispro	Aspart	IGF-I
HCT-116 proliferation at 96 hrs (compared to untreated cells)	+0.4%	+22%	+17%	-	-	+24%
HCT-116 proliferation at 48 hrs[1] (compared to untreated cells)	+7%	-	-	+20%	0	+22%
PC-3 proliferation at 72 hrs (compared to untreated cells)	+2%	+17%	+15%	-	-	+25%
MCF-7 proliferation at 72 hrs (compared to untreated cells)	0	+14%	+6%	-	-	+22%
HCT-116 % apoptotic cells at 12 hrs (control = 23%)	25%	15%	18%	-	-	17%
HCT-116 % apoptotic cells at 24 hrs (control = 30%)	30%	26%	25%	-	-	24%

[1] by MMT assay; all other proliferation experiments were measured by cell counts. Apoptotic cells were quantified via flow cytometry of Annexin V-FITC and Propidium Iodide labeled cells.

10 and 20 minutes of treatment, glargine phosphorylated both IR and IGF1R, and detemir phosphorylated IR but not IGF1R. Glargine further led to increased phosphorylation of both Akt and ERK, representing the 2 major signaling cascades of IR and IGF1R, without changes in the total protein amounts; phosphorylation was maximal at 20 minutes and decreased by 60 minutes. In a test of relative potencies, cells were treated for 30 minutes with each hormone at 50 ng/mL. Glargine and insulin both significantly increased the amount of phosphorylated Akt in comparison to untreated cells, while detemir and IGF-I did not significantly alter Akt phosphorylation. Insulin alone significantly increased ERK phosphorylation.

The authors concluded that at the supra-physiologic doses tested, glargine and detemir have significant IGF-I-like mitogenic activity, which is not shared by insulin. The authors' warning bears repeating: current evidence shows that neither IGF-I nor insulin (and hence, one would expect the insulin analogues as well) can cause malignant transformation. However, IGF-I does increase the aggressivity of already transformed cells. Thus, the question raised by this paper is whether long-term exposure to the insulin analogues can likewise affect cancer behavior.

Weinstein D, Simon M, Yehezkel E, Laron Z, Werner H. Insulin Analogues Display IGF-I-like mitogenic and anti-apoptotic activities in cultured cancer cells. Diabetes Metab Res Rev. 2009; 25:41-49.

Editor's Comment: *It would take a colossal leap to answer the underlying question based on the data of this pilot study. However, the results are intriguing enough to suggest more rigorous investigations are warranted. The high prevalence of both cancer and diabetes in our society, plus the widespread long-term use of these modified insulin analogues, makes the question an important one to answer. If—and this is a big if—it pans out that one or more of the insulin analogues is more stimulatory for cancer behavior, then cancer risk will become yet another factor clinicians must consider in selecting the particular insulin regimen for an individual patient.*

Adda Grimberg, MD

Continuous Glucose Monitoring and Intensive Treatment of Type 1 Diabetes

The Juvenile Diabetes Research Foundation (JDRF) Continuous Glucose Monitoring Study Group reported their findings of a multicenter clinical trial which randomly assigned 322 adults and children with type 1 diabetes to continuous glucose monitoring (CGM) or a control group which performed blood glucose monitoring with a glucose meter. All subjects were followed for 6 months to determine whether CGM helped to produce a sustained lowering of HbA1c and a reduction in hypoglycemia. The subjects were stratified by age: 8 to 14 years, 15 to 24 years, and over 25 years of age, and by HbA1c ≤8% and >8%. Individuals with HbA1c of <7% or >10% were excluded. Subjects had to be using an insulin infusion pump, or at least 3 daily insulin injections, to control their diabetes and could not have had experience with CGM for the 6 months prior to the trial. The final study group included subjects who used either the Dexcom 7® (Dexcom™), the Mini-Med Paradigm® Real Time Insulin Pump and Continuous Glucose Monitoring System (Medtronic), or the FreeStyle Navigator® (Abbott Diabetes Care) according to the manufacturer's instructions which included specific calibration procedures and replacement of the sensors every 3 to 7 days.

Subjects were instructed to verify the accuracy of CGM determinations with self blood glucose meters before making treatment decisions. Subjects were also given written instructions on how to use the data generated by the CGM and blood glucose meters to make real-time adjustments in insulin doses. Target pre-meal blood glucose values were identical for the study group and the control group, 70 to 130 mg/dL (3.9 to 7.2 mmol/L); target peak post-prandial values were <180 mg/dL (10 mmol/L), and bedtime overnight values 100 to 150 mg/dL (5.6 to 8.3 mmol/L). Subjects were seen at weeks 1, 4, 8, 13, 19 and 26 with one telephone contact between each visit to review glucose data and adjust diabetes management. After visits at 13 and 26 weeks, the control group used a blinded CGM for one week in order to compare continuous glucose profiles with the treated group. HbA1c was measured at 13 and 26 weeks and adverse events including severe hypoglycemia (defined as requiring assistance from another person and/or the use of glucagon), hyperglycemia with ketoacidosis, or other events were recorded.

The trial included 322 subjects (CGM group n=165; control group n=157); 114 patients were between 8 to 14 years of age (CGM group n=56, control group n=58), 100 subjects between 15 to 24 years of age (CGM group n=57, control group n=53) and 98 participants were over 25 years of age (CGM group n=52, control group n=46). A significant between group difference in the change in HbA1c from baseline to 26 weeks was seen in subjects who were 25 years of age or older, but not in those 15 to 24 years of age, or 8 to 14 years of age. In addition, in the CGM group over 25 years of age there were improvements in all measures of glycemic control including pre-meal and post peak-meal glucose values. The secondary analysis showed more patients in the CGM group had a reduction of 10% or more in mean HbA1c and more patients achieved their target HbA1c of <7.0%. Among subjects 15 to 24 years of age, the mean decrease in HbA1c from baseline to

26 weeks was 0.2% in both groups and among those 8 to 14 years of age the mean decrease was 0.37%. There were no statistical differences in the reduction in HbA1c between the CGM group and the control group for both of these ages. There were no significant differences in the incidence of severe hypoglycemic events between the CGM groups according to age; however, severe events were infrequent in both groups. Sensor use was greater among subjects 25 years of age or older with 83% of the subjects using the sensor at least 6 days/week. In the group 15 to 24 years of age 30% used the sensor 6 days/week, and in those 8 to 14 years of age 50% used the sensor 6 days/week. Sensor use was not associated with baseline HbA1c.

The JDRF Continuous Monitoring Study Group concluded that the benefit associated with CGM with regard to improved glycemic control was strongly related to age. Individuals greater than 25 years of age clearly benefitted while those 15 to 24 years of age did not benefit. Those 8 to 14 years of age had greater benefit than those 15 to 24 of age years. The authors further commented that before generalizing these results it is important to remember that all of the subjects in this trial were receiving intensive insulin therapy and that most of them had better than average HbA1c. Of note, the results for subjects using multiple daily injections were similar to the results of those using an insulin pump. The researchers further concluded that CGM may improve HbA1c and enhance the management of type 1 diabetes in adults who have the motivation to use the technology and incorporate it into their daily management.

Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group. Continuous glucose monitoring and intensive treatment of type 1 diabetes. N Engl J Med. 2008;359:1464-1476.

Editor's Comment: *Many pediatric endocrinologists have been waiting to see the data in this study regarding CGM. Many may see the results as disappointing, but maybe not surprising. Adults with strong motivation to use the CGM 6 days/week seem more likely to utilize the information to improve their glycemic control. Children 8 to 14 years of age–whose diabetes management is mostly directed by their parents–who also may have great motivation receive a greater benefit than adolescents 15 to 24 years of age, but not as much benefit as the adults. The results from the adolescents (who used CGM the least) is not surprising. CGM provides an incredible amount of real-time information regarding glycemia. For many people this information is overwhelming and of such a magnitude that organizing and responding to it is difficult. The JDRF study does not report any psychological, behavioral, or social information regarding the participants. Indeed such factors may have a great influence on subjects' ability to successfully manage their diabetes. It is hoped that such information was collected and that further reports of this data will include such information. Until such information is reported and correlated with the findings, the study remains incomplete.*

Pediatric endocrinologists still do not know for whom CGM will provide the greatest benefit and how such information can best be used by their patients. CGM most likely will not be widely used by the majority of persons with type 1 diabetes; but for a subset of individuals the information from CGM may greatly improve their ability to reduce glycemic variability and their risk of long-term complications.

William L. Clarke, MD

Primary Thyroid Carcinoma in Childhood Cancer Survivors

With modern therapies and supportive care, the number of the childhood cancer survivors (CCS) has increased considerably. However, these patients suffer from the late-onset complications such as endocrine impairments, neuropsychological problems and second malignancies. These late-onset complications often do not become clinically apparent until decades after therapy. Since the likelihood of follow-up decreases with time, it is important for physicians as well as patients and family members to be aware of the late-onset complications over their lifetime.

Patients who received upper-body radiotherapy for childhood cancer have an increased risk of developing primary thyroid cancer later in life. Brignardello et al set forth the recommendations for monitoring the late-onset complications of thyroid carcinoma by thyroid ultrasound screening into young adulthood, and beyond, in CCS. They observed a very high occurrence of thyroid carcinoma as a second malignant neoplasm in a total of 129 CCS who were previously treated with radiotherapy involving the head, neck, or upper thorax. The patients had had brain tumors, Hodgkin's disease, acute lymphoblastic leukemia and received preventive brain irradiation or total body irradiation for bone marrow transplantation. Thyroid ultrasound surveillance usually began 5 years after radiotherapy and was repeated every third year, if negative. Median follow-up time since the primary childhood cancer diagnosis was 15.8 years (range 6.1 to 34.8 years). Solid thyroid nodules were found in 35 patients included patients with palpable nodules (n=6) as well as those with solid nodules larger than 0.5 cm detected by thyroid ultrasound. Fourteen patients had nodules over 1 cm, 8 of which were not palpable. Fine-needle aspiration was performed in 19 patients, of which 14 had nodules over 1 cm. Cytological examination of specimens resulted in papillary carcinoma diagnosed in 5 patients and follicular carcinoma in 6 patients. In the remaining 8 patients, 7 had a diagnosis of nodular hyperplasia and one had lymphocytic thyroiditis. The

cytological diagnosis of papillary thyroid carcinoma was confirmed by histological examination in all 5 subjects who underwent surgery. Notably, only 2 of these patients had palpable nodules; the other 3 were smaller than 1 cm and were only detected by ultrasound. However, histological examination showed nodal metastases in 2 of them. In all 6 patients with follicular neoplasms who underwent surgery, the histological examination showed a benign lesion (goiter, n=3; follicular adenoma, n=3). Thyroid function was normal in 87 subjects, whereas 42 had primary hypothyroidism (n=37) or central hypothyroidism (n=5).

Brignardello E, Corrias A, Isolato G, et al. Ultrasound screening for thyroid carcinoma in childhood cancer survivors: A case series. J Clin Endocrinol Metab. 2008;93:4840-4843.

Editor's Comment: *This is a very interesting article; it provides important information for physicians who care for CCS. Because survival rates of childhood cancer patients have improved markedly in recent years, the risk of developing a thyroid neoplasm clearly increases over many years after radiation therapy involving the head, neck, or upper thorax during childhood. Brignardello et al reported the prevalence of thyroid cancer, thyroid nodules and other thyroid alterations increased in the long-term follow-up of CCS.*

There are 2 other papers on the subject worthy of discussion. In a 2003 retrospective study of all survivors of childhood and adolescent malignancies treated at Memorial Sloan-Kettering Cancer Center, Acharya et al[1] reported 33 patients who developed a clinically apparent thyroid neoplasm after therapeutic radiation. The median age at the time of diagnosis of the primary malignancy was 12.0 years (range, 3.7 to 18.3 years). The most common primary malignancy seen was Hodgkin's disease (n=18 patients), followed by non-Hodgkin's lymphoma (n=10 patients). The median interval from the time of radiation therapy until the recognition of thyroid disease was 13.0 years (range, 6.2 to 30.1 years). Thirteen of 33 thyroid lesions (39%) were malignant (11 papillary carcinomas and 2 follicular carcinomas). All thyroid abnormalities were detected on routine physical examination. Seventeen patients presented with a single nodule, 7 with multiple nodules, 5 with a multinodular goiter, 2 with lobar enlargement, 1 with a diffuse goiter, and 1 with an enlarged cervical lymph node and a normal thyroid gland. Thyroid ultrasound results were abnormal in 18 of 19 patients. Ultrasound revealed the presence of multiple nodules in 33% of patients, whereas only 15% of those patients had multiple nodules that were appreciated on physical examination.

In 2005, Sigurdson et al reported 72 cases with pathologically confirmed thyroid cancer from 14054 survivors (5 years or longer) of cancer during childhood from the Childhood Cancer Survivor Study cohort.[2] Childhood cancers were diagnosed between 1970 and 1986 with cohort follow-up to 2000. Of the 72 cases with secondary thyroid neoplasms, 56 (78%) were papillary, 11 (15%) follicular, and 5 (7%) of other or unspecified histology; 29 cases had a first diagnosis of Hodgkin's lymphoma and 14 had leukemia. They showed that the risk of subsequent primary thyroid cancer after a first tumor in childhood rose with increasing radiation dose (greatest risk 20–29 Gy), but decreased at doses of more than 30 Gy. Patients younger than 10 years at first cancer diagnosis had a higher risk of thyroid cancer than patients aged 10 years or older.

It is evident from these studies that thyroid nodules, even those greater than 1.5 cm, cannot always be palpated. In the study of Brignardello et al only 2 of the 5 patients with papillary thyroid carcinoma had palpable nodules. In the other 3 cases, the nodules were less than 1 cm, and were only detected by ultrasound. Therefore, the authors recommended monitoring the thyroid cancer by thyroid ultrasound screening in CCS previously treated by radiotherapy involving the head, neck, or upper thorax. Early detection of secondary thyroid cancers could improve the outcome of the patients. However, because thyroid ultrasound also detects many small lesions, the majority of which are benign, a very careful evaluation is needed to ascertain the results of thyroid ultrasound screening.

Brignardello et al emphasized the need for long-term follow-up for all CCS, which clearly must be extended well beyond childhood. Follow-up must

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address transitional strategies to avoid dropout and improve the overall outcome of childhood cancer treatment and survivors. Also it is necessary for physicians, as well as patients and family members, to know that late-onset complications of a cancer survivor can occur even after many years following cancer treatment.[3]

Yoshikazu Nishi, MD

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Renal and Urinary Tract Anomalies in Congenital Hypothyroidism

Newborn screening for congenital hypothyroidism (CH) is one of the major achievements of preventive medicine, as the condition occurs frequently (1/3000~4000 newborns). An early diagnosis and treatment prevents brain damage and the ensuing mental retardation. It is well known that CH has increased incidence of congenital malformations of heart, gastrointestinal, and skeletal systems. However, the prevalence of congenital renal and urologic anomalies on CH has not been well established.

Kumar et al reported that children with CH have significantly increased risk of congenital renal and urological anomalies. They investigated the prevalence of congenital renal and urologic anomalies in children with CH as compared to children without CH. Analysis of Congenital Malformation Registry data showed 980 children with CH and 3,661,585 children without CH born in New York State (1992-2005). Children with CH had a significantly increased risk of congenial renal and urological anomalies with the odds ratio (OR) of 13.2 (10.6-16.5). The other significantly increased defects and prevalence rates in patients with CH were cardiac, gastrointestinal, and skeletal (Table). Analysis of matched data (CH data from New York State newborn screening; 1,538 children with CH and 3,654,033 children without CH) also confirmed an increase of congenital renal and urologic anomalies with an OR of 4.8 (3.7-6.3). There are limitations of their study; the Congenital Malformation Registry is complied on the basis of hospital-generated data and is limited to children under 2 years of age. Therefore, there may be an underestimating of the true prevalence of congenital renal and urologic anomalies.

Hydronephrosis, UPJ obstruction, hypospadias, renal dysplasia, and renal agenesis were especially significant. Therefore, they suggested that CH children should be evaluated for the presence of congenital renal and urologic anomalies by a renal ultrasound examination.

Prevalence rates of congenital anomalies in hypothyroidism (CH) and in general population (non CH)

Congenital anomalies	CH (RATE/10 000)	Non-CH (RATE/10 000)
Renal		
Dysplastic kidney	30.6	1.7
Renal agenesis	102.0	4.3
Ectopic kidney	30.6	1.7
Hydronephrosis	346.9	21.1
Hydroureter	20.4	1.5
UPJ obstruction	30.6	1.9
Reflux	20.4	0.4
Hypospadias	275.5	39.6
Obstruction meatus	20.4	0.3
Posterior urethral valves	10.2	0.7
Cardiovascular		
Atrial septal defect	622.4	29.0
Ventricular septal defect	602.0	36.6
Coartation of aorta	81.6	4.1
Tetrology of Fallot	183.7	4.6
Endocardial cushion defect	275.5	3.1
Gastrointestinal		
Duodenal atresia/ stenosis	51.0	1.6
Gastroschisis	10.2	1.4
Omphalocele	40.8	1.3
Oral clefts	91.3	12.9
Pyloric stenosis	40.8	17.1
Tracheoesophageal fistual	61.2	2.4
Skeletal		
Craniosynostosis	50.0	4.0
Congenital hip dysplasia	30.6	1.7
Limb reduction	40.8	3.3

Modified from Kumar J, et al. J Pediatr. 2009;154:263-266.

Kumar J, Gordili R, Kaskel FJ, Druschel CM, Wordniecki RP. Increased prevalence of renal and urinary tract anomalies in children with congenital hypothyroidism. J Pediatr. 2009;154:263-266.

Editor's Comment: *This is a very interesting article; it provides important information for physicians who care for patients with CH and elucidates the high incidence of*

congenital renal and urologic anomalies. Early detection of these anomalies may prevent or delay the risk of renal damage and developing end-stage kidney disease. The paper also provides data regarding the prevalence and odd risk ratios of cardiovascular, gastrointestinal, and skeletal anomalies in CH.

The causes of CH are: thyroid agenesis or hypoplasia, which accounts for 20% to 40% of the cases; ectopic thyroid, which accounts for 45% to 60%; and dyshormonogenesis, which accounts for the remaining 10% to15% of cases. However, Kumar's observation did not discerned the association differences of congenital renal and urologic anomalies among these types of CH; they reported that mutations in PAX8, TITF1, and FOXE1 genes have been associated with CH in patients with either isolated thyroid dysplasia or thyroid dysplasia with associated malformations involving kidney, lung, forebrain, and palate.

Hydronephrosis was the major defect in CH while hypospadias was most seen in the general population. The renal and urologic anomalies except hypospadias are not found on a routine physical examination, but can be easily detected by a renal ultrasound examination. Hypospadias can be easily diagnosed on a routine physical examination. Therefore, they recommended a routine renal ultrasound examination in CH.

Yoshikazu Nishi, MD

Corticotropin-Releasing Hormone Testing in Assessment of Hypothalamic-Pituitary-Adrenal Axis Function in Infants with Congenital Central Hypothyroidism

The ACTH deficiency in neonates with multiple pituitary hormone deficiencies (MPHDs) results in sustained hypoglycemia and neuroglycopenia and is a major cause of morbidity and mortality. Under basal conditions, clinical signs of hypothalamus-pituitary-adrenocortex (HPA) axis dysfunction are usually absent and the HPA axis is probably the most difficult to assess in the neonate. For the assessment of HPA axis function in the neonate the corticotropin-releasing hormone (CRH) test (in which both the ACTH secretion by the pituitary gland and the subsequent cortisol secretion by the adrenal cortex can be evaluated) was considered as the most relevant choice. The overall aim of the study by van Tijn and colleagues was to develop a diagnostic workup for fast and reliable assessment of HPA axis function in neonates with congenital hypothyroidism of central origin (CH-C), detected by neonatal screening.

This was a Dutch nationwide prospective study (enrollment 1994–1996). Patients were included if neonatal CH screening results were indicative of CH-C and HPA axis function could be tested within 6 months of birth. Nine male and 3 female infants with CH-C and 4 infants with false-positive screening results or transient hypothyroidism were included in the study.

The assessment of HPA axis function was based on CRH and ACTH tests, multiple random plasma cortisol samples taken in the 24-hour period between thyrotropin-releasing hormone (TRH) and CRH tests, determination of cortisol excretion in 24-hour urine samples collected during this same interval, and long-term follow-up. For each patient the results of all endocrine examinations, including the other hypothalamic-pituitary axes, in combination with the results of cerebral MRI, added up to profiles on which overall diagnoses of HPA function were based. Diagnoses were reevaluated after 5 and 10 year follow-up (false positives, 3 to 5 year follow-up).

Of the 12 CH-C patients included in the overall analysis, 3 showed diminished peak responses to CRH of both ACTH and cortisol (subjects 1–3). In addition, their highest measured random plasma cortisol concentrations and 24-hour urine cortisol excretions were below the predefined cutoffs. Another 4 infants (subjects 4–6 and 12) showed adequate ACTH peak response, but diminished cortisol peak response. This discordant response was considered abnormal. All 4 subjects with false-positive screening results included in the overall analysis were diagnosed as having sufficient HPA axis.

The CRH test proved to be a fast and reliable tool in the assessment of HPA axis dysfunction in asymptomatic neonates at risk for serious morbidity and mortality when congenital hypothyroidism had been detected. The discordant response type with normal ACTH, but low cortisol response, which has not been described before, may be an early phase of HPA axis dysfunction. A prolonged follow-up until the age of 10 years in some patients confirmed the neonatal diagnosis and the choice of early hydrocortisone replacement therapy.

van Tijn DA, de Vijlder JJ, Vulsma T. Role of corticotropin-releasing hormone testing in assessment of hypothalamic-pituitary-adrenal axis function in infants with congenital central hypothyroidism. J Clin Endocrinol Metab. 2008;93:3794-3803.

Editor's Comment: *The cortisol peak response to CRH is the most valuable marker of HPA axis function. Ten years of follow-up have shown that it has the highest predictive value of all criteria evaluated in this study. In neonates with hypoglycemia and/or persistent jaundice, HPA deficiency can be suspected. However in the most cases there is no clinical indicator to avoid the high risk of death in early MPH deficiency. With the background provided by neonatal screening for hypothyroidism as suggested by the Dutch set-up[1] the CRH test appears to be the most valuable tool for early diagnosis of HPA axis dysfunction and for hydrocortisone treatment. As already known, hypothalamic-*

pituitary MRI would show in a large proportion of these cases; the most significant developmental abnormalities would be an ectopic posterior pituitary.

Raphaël Rappaport, MD

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Predictors of Relapse of Hyperthyroidism

There is debate about how Graves' disease (GD) should be treated in children. Remission is achieved in less than 30% of children treated with antithyroid drugs (ATD) vs 40% – 60% in adult patients. When relapse occurs, thyroidectomy or radioactive iodine treatment is considered, although the use of these therapeutic options in children remains controversial. Reliable predictors of relapse after ATD treatment would greatly improve patient management, by facilitating the identification of children requiring long-term ATD or needing early surgery or radioiodine therapy.

The aim of this study was to identify predictors of relapse after ATD treatment in children with GD. This was a prospective, multicenter cohort study of children (n=154) with GD treated with carbimazole for an intended duration of 24 ± 3 months. Most patients (n=147, 95%) completed 1 course of ATD. After the end of treatment, patients were followed up for at least 2 years. The primary outcome was hyperthyroidism relapse. Cox's regression analysis was used and a prognostic score was constructed.

Hyperthyroidism relapse was frequently observed after ATD treatment was stopped. The overall estimated relapse rate for hyperthyroidism was 59% (95% CI, 52% – 67%) at 1 year and 68% (95% CI, 60% – 76%) at 2 years after the end of ATD treatment. Median time to relapse was 8 months (95% CI, 5.4 to 11.4 months). In total, 87 of the 99 relapses occurred in the first year, principally in the first 6 months (n=64). Five variables were identified as independent predictors of relapse in a multivariate Cox model: age, serum free T_4 and TRAb levels at the time of diagnosis and duration of ATD treatment. Non-Caucasian patients were found to be 2.5 times more likely to suffer a relapse than Caucasian patients. Relapse risk decreased with increasing age at onset (hazard ratio [HR] = 0.74 per 5 year increase in age, P = 0.03) and duration of first course of ATD (HR = 0.57 per 12 months, P = 0.005). A prognostic score was constructed, allowing the identification of 3 different risk groups, with 2-year relapse rates of 46%, 77%, and 98% (Table). Overall, marked differences in the observed and predicted relapse rates were found among the 3 identified risk groups. The patients in risk group A had a predicted 2-year relapse rate of 46%, whereas those in group C had relapse rates as high as 98% at 2 years after the end of ATD treatment.

Prognostic score for relapse in children with GD[1]

Weight	0	1	2	3
Ethnicity	Caucasian		Non-Caucasian	
Age	>12 years	1-12 years	<5 years	
Free T_4 serum concentration	<50 pmol/L			≥50 pmol/L
Multiple of upper normal limit for TRAb concentration	≤x4(N)2	>x4(N)2		
Duration of ATD treatment	>24 months			≤24 months

For each patient, score may range from 0 to 11.

[1] The prognostic score was calculated from the data of 138 of 147 patients because of missing data (n=9).

In conclusion, this study, which is, to our knowledge, the largest prospective study in children with GD, provided strong evidence that there is an association between ethnicity, age, and disease severity at diagnosis and the risk of relapse 2 years after the end of the initial course of ATD treatment. Results suggested that the use of prolonged courses of ATD treatment is associated with a better outcome. Indeed, the duration of medical treatment seems to be the only variable related to risk of relapse that can be manipulated, as every additional year of treatment was associated with a decrease in relapse rate. The use of a predictive score, with treatment duration adjusted as a function of the patient's characteristics, to improve the prognosis could have important implications in daily practice and should be validated by application to another population of children with GD.

Kaguelidou F, Alberti C, Castanet M, Guitteny M-A, Czernichow P, Léger J for the French Childhood Graves' Disease Study Group. Predictors of autoimmune hyperthyroidism relapse in children after discontinuation of antithyroid drug treatment. J Clin Endocrinol Metab. 2008;93:3817-3826.

First Editor's Comment: *Although radioiodine or surgery have been advocated as the first choice of therapy in children with autoimmune hyperthyroidism, ATD therapy remains the first choice in most clinics. Therefore, this prospective paper deserves much attention. The study was carefully managed and most of its methodological limitations were taken into account. Because it is everyone's experience that the outcome is rather unpredictable, these data with a practical scoring may turn out to be quite*

useful in the management of individual cases and with the difficult task of maintaining compliance. It also made it possible to identify a small group of children at a very high risk of relapse, essentially young (<5 years of age) non-Caucasian children with severe initial hyperthyroidism. In conclusion, a longer initial duration of a euthyroid state with ATD treatment is the most significant prognostic variable. However, the optimal duration remains to be evaluated in further studies.

Raphaël Rappaport, MD

Second Editor's Comment: *Hyperthyroidism is believed to result from a complex interaction between the autoimmune system, environmental factors, and genetic background; it is mainly due to Graves' disease and is less frequently seen in children than in adults. ATD treatment is the initial form of therapy for all hyperthyroid children in an attempt to normalize thyroid function tests. Whether this form of treatment is continued long-term or whether other therapeutic options, such as surgery or radioactive iodine treatment, are considered is often dependant on the rate of relapse after ATD treatment. Reliable predictors of relapse after ATD treatment would facilitate the management of these children by allowing for the identification of those requiring long-term ATD, or alternatively thyroidectomy or radioiodine therapy.[1] In this study, Kaguelidou et al were able to find the 5 variables most predictive of relapse following ATD. At diagnosis the key factors to consider when evaluating the risk of relapse of a patient are: ethnicity (higher risk for children of Non-Caucasian origin), age (the younger the patients the higher the risk for relapse), severity of the disease as manifested by elevated serum free T_4 and TRAb levels (the higher these concentrations, the higher the risk of relapse) and duration of the disease. It is interesting to note that children receiving longer ATD treatment were less likely to relapse, with a 43% decrease in relapse risk for each additional 12 months of treatment. While it is clear that there is no ideal form of therapy for this disease as the 3 available therapeutical options (ATD, thyroidectomy, and radioactive iodine) are associated with potential complications, drug therapy remains the first line of treatment in many countries. The remission rate after 2 years of ATD treatment (about 30%) observed in this study is in agreement with a 1987 report.[2] This study also demonstrated that the remission rate increases significantly in children and adolescents with every additional year of treatment. The need for prescribing longer treatment courses in children than in adults is now widely accepted and the duration of medical treatment seems to be the only variable, independent of ethnicity, age and severity of disease, that can be manipulated.*

Roberto Lanes, MD

Third Editor's Comment: *The value of predictors to determine the relapse risk of patients with hyperthyroidism following ATD therapy has long been studied in children and adults. The most controversial factor is the serum TRAb level which may not be sufficiently sensitive to predict a relapse after ATD treatment[3] even though others have considered them useful in children.[4] TRAb data are often lacking at diagnosis and/or during follow-up of these patients with Graves' disease, as was the case in this study of Kaguelidou et al. However, the long-term results of ATD treatment remain generally unsatisfactory in most studies. Poor compliance with medical therapy is often the most important factor that determines the therapeutic outcome, particularly in adolescents. Yet long-term treatment seems to be the only variable that is at the clinician's control to reduce the risk of relapse of the disease. Thus, important arguments have been put forward for considering ^{131}Iodine therapy or surgical ablation in the treatment of children with hyperthyroidism.[5,6]*

Fima Lifshitz, MD

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Treatment Guidelines for Children with Disorders of Sex Development

Disorders of sex development (DSD) is the umbrella term replacing intersexuality to cover congenital conditions characterized by atypical chromosomal, gonadal, or anatomic sex.[1] This article was published in a special issue of a journal focusing on gender identity disorders (GID). However, Meyer-Bahlburg sees sufficient differences between gender-variant persons, with and without a DSD, to urge distinct evaluation and treatment approaches.

GID is characterized by discomfort or distress with one's apparent or assigned gender accompanied by a persistent identification with the opposite sex. In

contrast, gender issues may be far less salient for those with DSD. The challenges related to having a chronic (for some, a life-threatening) medical condition, and its associated management, require that the behavioral health professional be competent in the application of psychosocial interventions for problems of medical adherence and in coping with the stigma often associated with congenital or chronic conditions, in general.

Whereas the evaluation and psychosocial treatment of persons with GID can be conducted by a mental health provider alone, the 2005 consensus statement on DSD[1] calls for care to be provided in the context of a multidisciplinary team, including: neonatology, pediatric endocrinology, pediatric urology, gynecology, genetics, genetic counseling, mental health specialists, social work, nursing, and medical ethics. Moreover, communication between the DSD team and the "medical home" (ie, the primary care physician) is strongly recommended. Because of the high stress at the time of ascertainment of the DSD, there is a critical need for good communication among team members and the family to facilitate shared decision making regarding gender assignment and surgical options. Information shared with the family must include the most up-to-date information regarding the patient's specific syndrome, including the range of prognostic outcomes for both physical health and psychological health across the lifespan.

Meyer-Bahlburg describes in considerable detail the complexities of the process of gender assignment, and potential reassignment, for those diagnosed at birth and the long-term risk for the child and family associated with missteps in clinical management. Providing integrated interdisciplinary team care for patients is complex and time-consuming for patients, in general. In the case of DSD, Meyer-Bahlburg notes the importance of sustaining the team approach beyond the period of initial diagnosis and early interventions. Optimal care also requires active outreach by the behavioral health provider of the team to adopt a preventive approach regarding problems with psychosocial adaptation and medical adherence. Children and their families may require assistance in interpreting gender-atypical behavior – a not infrequent occurrence in DSD – as understandable based on what is known of the biology of DSD, rather than as a sign of incorrect gender assignment.

Young children with a DSD, who were misdiagnosed or late diagnosed, create special challenges. Parents may require reassurance and counseling if gender atypical behavior is part of the presentation. Gender reassignment after infancy requires careful psychological evaluation over a prolonged period with particular attention to the child's gender-role behavior and to any symptoms of gender dysphoria.

There is no controversy over performing genital surgery for acute medical reasons. However, medical urgency is the exception rather than the rule. Instead, genital surgery has been performed, typically early in life, to "confirm the assigned gender by genital appearance." At present, there is no broad consensus regarding the issue of early genital surgery, with the exception of the milder cases of atypical genital development for which deferring surgery is now recommended.[1] Meyer-Bahlburg describes the preparation required for parents and, later, patients regarding genital surgery decisions. The psychological risks for the patient associated with repeated genital examinations, in part to support the training of medical students and residents, requires a rethinking of medical educational models and practices.

This article provides additional guidance regarding androgen treatment in 46,XY children with underdeveloped genitalia and the timing of sex hormone treatment in persons without gonads or with under-functioning gonads. In general, the timing of hormone replacement is best initiated during the period when peers are experiencing endogenous puberty.

The topic of disclosure of medical information to the patient is a crucial component of psychosocial management. Although legal standards generally support the rights of parents to determine what and when details of their child's medical condition is disclosed to them, the majority of clinicians agree that a patient with a DSD be fully informed of all details. Some parents will resist disclosing information to their child or adolescent, in particular in cases in which the assigned gender is at odds with sex chromosomes or gonadal structure. Parents can be reassured by the experience of patients with other medical conditions (eg, pediatric cancer or HIV) that disclosure is associated with enhanced psychosocial adaptation. Meyer-Bahlburg reviews strategies for the disclosure process, including providing a web link to a source for animated visual aids.

Finally, the beneficial role of support groups for persons with medical conditions, in particular for those with rare conditions, is emphasized. Health care providers are encouraged to seek opportunities to dialogue with such groups to ensure that the information disseminated is accurate.

Meyer-Bahlburg HFL. Treatment guidelines for children with disorders of sex development. Neuropsychiatrie de l'Enfance et de l'Adolescence 2008; 56:345-349.

Editor's Comment: *Important distinctions between persons with GID and DSD are often blurred in both the popular and scientific literature. While the entities may share some features (eg, gender concerns), DSD diverge from GID in terms of associated features including prevalence, age of onset, and sex ratio.[2] Failure to differentiate between persons with and without a clearly identifiable DSD may hamper studies of etiology and optimal clinical management.*

The recently published consensus on the management of DSD[1] is very clear on the necessity of applying an integrated interdisciplinary team approach to the care of those affected and their families. What

is not discussed are the barriers that exist to forming and sustaining such teams. The non-reimbursable time required to organize a team is substantial and the essential behavioral health component of service is frequently excluded because clinical services provided by mental health providers are often carved-out by many health insurance plans and require the patient to be seen by an approved insurance panel member who would not be a member of the DSD team. Co-pays for mental health services are characteristically substantially higher than for medical services, forcing families to reject recommended services delivered by the behavioral health member of the team. At present, there is a scarcity of behavioral health experts qualified to immediately join emerging DSD teams. However, the skills set of pediatric psychology makes this subspecialty of child clinical psychology ideally suited to serve as team members at centers of excellence called for in the DSD consensus statement.[1]

An evidence-based consensus on best clinical practices regarding gender assignment and genital surgery is only beginning to emerge; in the meantime there is critical need for systematic investigation to understand how parents are counseled and select among treatment options. Clinicians and representatives of patient advocacy organizations voice concerns about the extent and quality of information disclosed to parents during the decision-making process and, importantly, the subsequent validity of parental consent. These factors make this an excellent clinical context in which to study parental medical decision-making.

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